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In Memory Of

CHARLES ALDERSON KELSALL

and

LUCY KATHERINE CRABB

PREFACE

There are a number of free or independent connective tissue cells whose functions have not been established Among these cells the lymphocytes plasmacytes and mast cells are probably the most significant, because of their relatively great numbers and well known capacity for densely ' infiltrating a particular area Together with eosinophils neutrophils monocytes gliocytes and various less obvious cells the mast cells lymphocytes and plasmacytes are considered to be very important in intermediary metabolism Certain of these trephocytes function directly in metabolism of other cells, but others may control the passage of nutritive material from the capillaries to the individual cells and the elimination of their products, as well as affecting the salt water balance in the tissues

The lymphocyte is a specialized cell which synthesizes stores and transports nucleoprotein for maintenance growth or secretion by other cells The plasmacyte is considered to be a synthesizing and storage cell for ribonucleic acid (RNA) and gamma globulin instead of having only the delimited function of antibody formation The functions of lymphoid tissues in the intracellular storage and release of protein is related to the situation of the particular lymphoid structure with regard to the immediate source of available protein Thus, the formation of lymphocytes in the spleen intestinal lymphoid tissue and lymph nodes has similar relations to the over all problem of normal and pathological protein anabolism The spleen is located between the systemic and hepatic portal blood vessels so that it may control the supply of plasma protein passing from peripheral blood to the liver by intracellular storage and release Likewise the intestinal lymphoid tissue, including the core of the villi may store some of the alimentary protein intracellularly, and aid in regulating the supply of protein for use by the liver Peripheral lymph nodes resynthesize extracellular protein from tissue fluid to the intracellular form in lymphocytes and plasmacytes and thereby, aid in regulating the level of plasma protein

The chief significance of the mast cell is the result of its ability to synthesize store and release histamine and heparin but its production of mucopolysaccharide (MPS) and its possible relation hip with erotonin is al o discussed Thus the mast cell plays an important part in the control of the permeability of blood and lymph capillaries and in diffusion through the collagenous connective tissue ground substance Mast cells effect changes in intercellular fluids by releasing histamine which produces hyperemia with increased capillary permeability

The number of trephocytes (feeding cells) and their rate of secretion or lysis forms an essential factor in the control of conditions in the recipient cells and/or in the intercellular fluids changes in the physiochemistry of

which markedly alter the metabolism of the various cells The activity of trephocytes is variously controlled by hormones, histamine epinephrine balance and the parasympathetic sympathetic nervous system In turn, the formation and disintegration of these cells is considered to form an intermediary step and mechanism which increase the delicacy of control in the various systems of balance and counterbalance

Ideas and results used for developing working hypotheses for these cells have been taken from diverse fields of the biological sciences including anatomy bacteriology, biochemistry, cytology, embryology, enzymic chemistry, medicine, veterinary medicine, parasitology and pharmacology

We do not profess to have covered the literature with the object of producing a bibliographical review Rather we attempted to incorporate the various ideas on, and different experimental approaches to the various aspects of the problems involved in this volume References gleaned from lists of literature cited, bibliographies and international indexing and abstracting journals were carded and evaluated The works deemed essential for these papers, but not available in the various libraries of the University of Colorado or in our personal collection of reprints, were borrowed through Library Interloan By these procedures we had access to works published in foreign countries as well as those published in this country The sources of abstracts used in this work are credited in the respective references in the list of Literature Cited

We have freely employed the 'historical citation to indicate who made the original statement and when it was made The historical citation is designated by the year of publication enclosed in parentheses immediately following the name of the author Historical references are not listed in the Literature Cited since we did not see the original work Authority for the statement is cited by a superior figure which indicates the reference listed in the Literature Cited

The term plasmacyte ' is used in this work instead of the more popular term 'plasma cell ' to conform with the rules formulated in 1948 by 32 members of the American Society of Clinical Pathologists who compiled the ' first report of the committee for clarification of the nomenclature of cells and diseases of the blood and blood forming organs (Committee 1948) Use and/or spelling of certain other terms are variable primarily because of our inability to find a definite rule or authority to follow For example both prefixes deoxy and 'desoxy are in common usage and occur in both American and European journals and books The abbreviation DNA is consistently used in English publications (ADN in French) for de or desoxyribo or de or desoxyribosenucleic acid We have however at the risk of censure by certain organic chemists limited the use of

the abbreviation AMP to designate acid mucopolysaccharide—never adenosine monophosphate

Part of this work, particularly that pertaining to the distribution and/or weight of lymphoid tissue[201–205] the effects of benzene poisoning splenectomy and/or appendectomy on lymphoid tissues and circulating leukocytes[512–514] the histogenesis of lymph nodules in the rabbit s appendix[201–207] and a comparative study of cecae[203–204] was begun during 1938 to 1943—and amplified and extended during the intervening years to 1958 Consequently, we are indebted to a number of individuals and organizations for various favors We are particularly indebted to various individuals of the U S Wild Life Service and the Henderson Museum of the University of Colorado for supplying the viscera of wild rodents, shrews and a few other mammals This work could never have been completed without the splendid cooperation and assistance of our assistants in research typists and photographers, and of the staffs of the Libraries and Research Services Laboratory of the University of Colorado We are especially indebted to Mrs Madeline L Atwell for her continued assistance with our research during the last 5 years and for her help with the many problems involved in the preparation of this manuscript

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Introduction

Lymphocytes plasmacytes, mast cells and certain other cells have functions in supplying nutritive materials and in regulating protein metabolism These cells are designated by the general term trephocytes (feeding cells) since they exist primarily for the benefit of other cells The nutritional relations of trephocytes with recipient cells commonly involves the interrelations between extracellular and intracellular metabolism of both the trephocyte and the recipient cell

The idea that living cells function in the nutrition of other cells and structures began more than a century ago with the work of Goodsir and Goodsir[337] Cameron[13] states that these investigators identified certain minute cellular parts, such as nuclei, which exist in the textures and organs as centers of nutrition, and that they concluded that "they draw from the capillaries or other sources of nutritive materials and distribute them to each organ or texture after its kind They also behave as centers of germination, the so called germinal spots Although their statements are not incisive, it is clear that the brothers Goodsir had a nebulous concept of trephocytes The idea that some cells of the body carry nutritive material was reported by Ranvier (1875 to 1900) who noted the phagocytic properties of free cells in loose connective tissue and in the omentum, and called them "clasmatocytes (which he believes are derived from extravascular lymphocytes) because 'they carry nutritive material which they deposit by breaking off protoplasm "[135]

This work sets forth the trephocytic activities of lymphocytes plasmacytes and mast cells However it is not the writers desire to promote the idea that trephocytes are all important In the final analysis, the plasma is the primary source of most substances utilized in intermediary metabolism whereas nucleocytoplasmic interrelations and the intracellular organelles are vital in the synthesis and retention of proteins These intracellular functions have been considered in detail by many investigators but the importance of lymphocytes and mast cells in regulating processes in the intercellular fluids has not been recognized

The interrelations between extracellular fluid and intracellular metabolism of most organs is generally recognized For example the relationship between decreased cytoplasmic basophilia in hepatic cells and decreased

leasing histamine to produce local hyperemia an increased capillary permeability (Chap 5)

Lymphocytes plasmacytes mast cells and other trephocytes are situated in direct contact with blood lymph or tissue fluid and have the function of synthesizing and storing various substances obtained from this fluid to be released for the regulation of metabolism in other cells These trephocytes retain the stored substances until they are freed Release of the trephones is controlled by alterations in hormones, by changes in the histamine epinephrine or in sympathetic parasympathetic balance The processes of withdrawal of amino acids and other substances from body fluids for synthesis and intracellular retention by trephocytes and the controlled release of these stored substances form a delicate mechanism which plays an important part in the regulation of protein synthesis, normal body growth repair and maintenance Furthermore, this mechanism permits the regulation of physiological processes at specific loci or in specific structures and facilitates the maintenance of function, regeneration and wound healing

TREPHOCYTES IN GENERAL

Generally trephocytes are considered to be heterogeneous non endocrine cells which synthesize and store nutritive regulative or other essential substances (trephones) for physiological processes This concept includes a number of cells in addition to lymphocytes plasmacytes and mast cells which have variously specialized multiple or little understood functions Lymphocytes and plasmacytes are pre eminently concerned with nucleoprotein metabolism, mast cells contribute histamine hyaluronic acid and polysaccharides to form heparin, cartilage basement membrane and probably other substances A considerable amount of material has been brought together in this chapter chiefly to stress the fact that lymphocytes and plasmacytes are not the only trephocytes which supply nucleoproteins

Lymphocytes plasmacytes and mast cells form an important mechanism for regulating local protein synthesis in an area such as in wound healing and also in more widely dissimilar pathological growth processes The time interval in relation to the sequence of steps is a crucial factor in considering the working hypothesis that these cells regulate protein metabolism in delimited areas The release of histamine and heparin by mast cells is a trigger mechanism in the sequence of steps in producing hyperemia and increased capillary permeability in a particular area Thus the significance of the mast cell in normal and pathological conditions can be interpreted only by considering the relation of the various stages in a particular process because mast cells may be present during one stage and

plasma proteins has been generally accepted Also it is generally conceded that all cells contain a certain amount of stored or reserve protein for the use of the individual cell, rather than for the use of other cells or as a contribution to the plasma protein as is the case of trephocytes

There are various cells and certain structures in higher vertebrates, the functions of which have not been definitely established Some of these cells retain as well as synthesize, various substances Release of stored substances from a cell is controlled by various agents, and the order of release from the different kinds of trephocytes forms a means of regulating protein synthesis The full significance of a single kind of trephocyte can be interpreted only in terms of its relation to other cells Trephocytes function as an intermediary between extracellular fluids and metabolism of fixed cells and the necessity for trephocytes is usually related to the nature of the intercellular material and to the presence or absence of motility and/or mobility of the recipient cell

A review of the literature indicates that large and small lymphocytes, plasmacytes and mast cells have multiple and varied functions Lymphocytes are the most versatile of all the trephocytes because of their general distribution, mobility motility great numbers rapid turnover and ability to assume additional roles by transforming into plasmacytes [34] Mast cells function chiefly in controlling the metabolic relations of plasma and intercellular fluid to intermediary metabolism by releasing substances which cause hyperemia and increased permeability of capillaries and connective tissue ground substance

The process of protein binding is also a factor in the transportation and utilization of substances in intermediary metabolism The linkage or other attachment of some substances to the globulins instead of albumins, could be an adaptation for regulating the distribution of the attached substance because there are differences in shape and size of the globulin molecules and in the ease with which globulin and albumin pass through the walls of capillaries The binding of various metals with plasma proteins increases the size of the molecule and may be a means of retaining these substances especially within the capillaries and in the blood sinusoids of the spleen and bone marrow The B_1 globulins contain copper and iron binding proteins Schade and Caroline (1946) report that B_1 globulin can bind two atoms of Fe which then has a molecular weight of 90 000 [13] The combination of thyroxine (mol wt 776 93) [694] as iodized protein in the form of thyroglobulin (mol wt 640 000) [1084] is an example of this increase in size of the molecule which might facilitate retention within the thyroid follicle [1084] There is evidence to indicate that mast cells function in promoting the local escape of these and other large storage protein molecules by re

combine several essential components for certain types of processes enhances its value Breaking a linkage such as that between stored histamine and heparin in granules of mast cells, is advantageous because histamine increases capillary permeability and heparin deters coagulation The linkage between histamine and heparin facilitates storage of these two powerful and highly active physiological substances by rendering them inactive Furthermore glucuronic acid, glucosamine and sulfuric acid groups may be released from heparin or other forms of AMP In turn, these substances may be related to AMP metabolism of other cells, such as mucinogen secreting cells, or to detoxication of substances in localized areas

The sequence of formation and lysis of the free connective tissue cells is neither clear cut or quantitative, nor do the same time intervals obtain Similarly, there are fluctuations in time intervals and in the quantity of various substances in the tissue fluid of localized areas For example, the intracellular storage of protein during the transformation of lymphocytes into plasmacytes follows a lapse of time during which there is a significant increase in extracellular protein as a result of capillary leakage The formation of plasmacytes is related to lymphocytic infiltration and the availability of protein in the extracellular fluid In turn, extracellular protein depends upon (1) increased capillary permeability and the duration of capillary leakage (2) the number of necrotic cells (3) the number of extravasated erythrocytes and other blood cells, (4) the number of infiltrating lymphocytes and (5) the retention of these cells and other substances by the fibrin network Disintegration of most of the plasmacytes occurs during the period of growth of the replacement tissue However in subchronic inflammation the causative factor is intermittently effective and consequently, the replacement process is prolonged Therefore more plasmacytes are formed because there is repeated exudation of proteins and longer intervals of time than those that occur in a process such as healing by first intention

The number of mast cells in muciferous glands is also related to the extent of the ground substance underlying the mucigen secreting alveolar cells In simple epithelium, such as the lining of the flask shaped mucous glands in the rabbit appendix[20] where most of the cells are secreting mucigen the underlying connective tissue usually has relatively few mast cells However the connective tissue adjacent to other muciferous glands such as the sublingual salivary and Brunner s glands in the hamster contains many disintegrating mast cells[5,7,6,8] These mast cells may be an important source of AMP and by this means have a direct relation to the mucin secreting alveoli[5,7,6,9] The primary function of the mast granules or their components released from the mast cells associated with Brunner s and the sublingual salivary glands, may be to release histamine to provide for in

absent during another Mast cells are most conspicuous between their periods of trephocytic activity because at this time they are synthesizing and storing substances to be supplied to other cells later Thus, the effects of these cells are not obvious during the stage in which they are most abundant, and, as the contents of their granules are released, they lose their identity

Substances released by mast cells may facilitate protein synthesis in a structure within an organ, such as the islands of Langerhans, within an organ of a system such as in the mammary gland during lactation, or in a delimited area of an organ, such as a lobe of a gland Mast cells play a significant part in the regulation of normal processes, especially those which are intermittent in function The salivary glands and capillary beds, for example function intermittently, that is they are subject to periods of hyperactivity and of hypoactivity Mast cells, lymphocytes and plasmacytes store in an inactive form, substances which are available almost instantly and in appropriate quantities during periods of activity of these organs The mast cells release histamine to increase capillary permeability and to obtain a readily available supply of plasma, as well as enzymes proteins and other substances from the blood, for local use Acid mucopolysaccharides (AMP) from mast granules may furnish substances for the secretion of mucin Lymphocytes and plasmacytes release concentrated nucleoprotein by dialysis, budding or lysis and thus facilitate protein synthesis in alveolar cells during periods between active secretion, at which time capillaries are not usually dilated Formation of plasmacytes from lymphocytes in the connective tissue around alveolar cells indicates that the transforming cells are retaining protein for later use by alveolar cells, possibly while capillary permeability is not being increased Thus in the salivary glands, plasmacytes and lymphocytes increase components for protein synthesis in active alveolar cells

Principles Involved

The formation and ladening of free connective tissue cells with substances for use by other cells is just as important as the disintegration of these cells and the release of the stored trephones The formation of trephocytes within connective tissue in contrast to those which are blood borne gives them optimal opportunity to regulate localized growth or physiological processes For example the formation of granules in mast cells represents the synthesis and storage of histamine heparin possibly serotonin and/or other substances Also it represents the withdrawal of substances from the extracellular fluid where they are not readily controlled to intracellular and inactive storage combinations which are more easily regulated and controlled Also the ability of a synthesizing and storing cell to

combine several essential components for certain types of processes enhances its value. Breaking a linkage such as that between stored histamine and heparin in granules of mast cells, is advantageous because histamine increases capillary permeability and heparin deters coagulation. The linkage between histamine and heparin facilitates storage of these two powerful and highly active physiological substances by rendering them inactive. Furthermore, glucuronic acid, glucosamine and sulfuric acid groups may be released from heparin or other forms of AMP. In turn, these substances may be related to AMP metabolism of other cells, such as mucinogen secreting cells, or to detoxication of substances in localized areas.

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cleotides, enzymes and other substances as forming a very important part of the trephocytic contribution (trephones) to recipient cells However, it is reasonably safe to assume that these functional processes involve extremely delicate and complicated chemical changes mediated to a great extent by inorganic catalysts, coenzymes, enzymes, ions and changing osmotic pressures An outstanding modification of this rule is manifested by those oocytes which engulf and digest their nurse cells

The general relations of some trephocytes to their recipient cells are well understood such as ovarian follicular cells and the recipient oocyte, others, such as phagocytes and erythrocytes, are best known for their specialized functions with little consideration being given to their trephocytic functions Interstitial cells of gonads are considered to be trephocytes [83] endocrine cells[493] or special phagocytes [493] The trephocytic nature of other cells such as microgial cells has been overlooked by many investigators All cells exert a certain amount of chemical regulative or other effect upon other cells, therefore, it is apparent that any functional classification of trephocytes is only empirical and that it is impossible to formulate a plan of classification of cells on a physiological basis which would be free from serious objections Trephocytes have multiple functions and some supply various substrates and catalysts, *e g*, manganese, magnesium and phosphorus and enzymes, *e g*, deoxyribonuclease (DNAase), hyaluronidase, nucleophosphatase, nucleosidase, nucleotidase, thymonucleodepolymerase and ribonuclease (RNAase)

The quantity of substance supplied is relatively great in some instances, as in the case of egg nurse cells or follicular cells However an infinitesimally small quantity of substance may be most important in converting certain nucleic or cytoplasmic substances into deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or adenosine triphosphate (ATP) or in initiating the utilization of phosphorus in synthesizing various proteins polysaccharides and other substances essential to the physiological processes of the cell organ or organism

Most cells may release certain amounts of growth promoting substances at death Thus the fact that a cell is dying does not in itself mean that its substances especially RNA, are not available to other viable cells [07] Indeed Marchand (1901) suggested that damaged tissue liberates 'growth promoting substances and several later workers support this idea [135, 87] Guterz (1925) attributed this growth stimulating function to necrohormones produced by degenerating nuclei [135] Montefoschi[709] believes that products of cell degeneration are always rich in RNA ' Englert [87] suggests that the active factors in proliferation promoting substances released by injured cells are "partly due to hydrolytic products of nucleide acid ' which

creased capillary permeability because these glands secrete intermittently, rather than continuously, as do the goblet cells Other mucigen secreting glands, which are characterized by the presence of very little connective tissue between the muciferous alveoli, also contain many mast cells This rule applies to the mucoserous glands in the tongue, olfactory membranes and trachea, which are similar to the paired salivary glands of the hamster

The greater the availability of AMP in the connective tissue ground substance, the fewer the mast cells The lacrimal is a serous gland and contains very few mast cells The glands of Krause in the eyelid contain more mast cells than the parotid or submandibular, but relatively fewer than the sublingual glands in hamsters This is correlated with the fact that, although the glands of Krause secrete some mucus it is in a dilute form Histologically Krause's gland is a low form of seromucoid gland, whereas the sublingual salivary is almost a purely mucinous gland in hamsters[5,7] Each secreting cell in Krause s glands is the same because there are no areas *in the alveoli which are exclusively mucus or serum secreting However* the main source of the AMP in connective tissues is fibroblastic cells

The various kinds of trephocytic cells also differ in mobility and motility Mobile and motile protein bearing trephocytes include small lymphocytes which occur in all body fluids Tissue mast cells probably have restricted motility but are not mobile Plasmacytes are between these two groups because they are not permanently attached and are sometimes mobile and may be slightly motile but are not normally present in circulating blood In addition to being a source of the amino acids of plasma proteins lymphocytes and plasmacytes play an important part as trephocytes by supplying essential trephones such as nucleic acids histones, magnesium phosphorus and certain catalyzing enzymes Some of these substances would probably be degradated destroyed disseminated or otherwise lost if the recipient cells had to depend entirely upon the plasma and tissue fluids for their delivery The expected result would be that these substances could not be assembled in the proper proportions and amount at the critical time and site Combination of these substances as a packaged unit in the lymphocytes permits their transportation to the area and their release at the site where and when needed

Action of Trephocytes

The physiochemical methods by which the various kinds of trephocytes perform their functions are not well known Some trephocytes release their trephones directly into tissue or circulating fluids whereas the trephones of others may pass directly into the recipient cell Histochemical and fluorescent methods have enabled investigators to identify nucleic acids, nu

and that lymphocytes do not[186] However, the above relations could also be interpreted to indicate that the plasmacytes contained more of the fluo rescein labeled antibody because it is a storage, as well as a synthesizing cell The possibility is not eliminated that lymphocytes may also be a source of antibody which is immediately removed from this cell by cytoplasmic budding or other mechanisms

Increases in the number of causative and reactive cells may appear to parallel each other For example, eosinophils have been considered a source of histamine because conditions in which histamine increases are usually accompanied by eosinophilia[8] When the histamine content is lowered, the number of eosinophils decreases These apparent cause and effect relations do not prove that eosinophils are a source of histamine Rather, they sug gest its disposal by eosinophils[5, 855, 105, 10] If eosinophils do have a function in destroying histamine as Vaughn[105] believes, eosinophilia would occur in most stages of increased histamine except the very earliest stage Thereby, a decrease in eosinophils would appear to parallel the fall in histamine

The histochemical characteristics of some trephocytes are not evident by the usual fixation and staining methods For example, the mast cell can not be identified by the usual methods of fixation because its basophilic granules which are characteristic of this cell, are readily soluble Changes in mast cells are seldom mentioned in de criptions of skin lesions except in urticaria pigmentosa xeroderma pigmentosa, and lupus erythematosis in lesions of which mast cells are greatly increased[4]

A more readily obvious function of a cell may overshadow an incidental or obscure function The obvious function of erythrocytes is transportation of oxygen but disintegration of dead erythrocytes in the spleen and bone marrow is a factor in protein metaboli m Phagocytosis by monocytes histiocytes and Kupffer cells is easily demonstrated by methods which have been in use for many years However, function of these cells in protein syn thesis and storage had to await the development of more recently devised biochemical and biophysical methods The function of astrocytes as sup porting cells in the central nervous system has been chiefly deduced from the striking morphological characteristics whereas the biochemical character istics apparently have not been studied in detail

Interpretation of the significance of structures composed primarily of lymphoid tissue requires consideration of their relation to body fluids The lymph nodes and the spleen contain reticuloendothelial cells which are filters of various body fluids However the reticuloendothelial (littoral) cells in the spleen are interposed to filter out bacteria certain parasites blood corpuscles protein and other substances in the systemic blood The Kupffer cells in the liver function as a similar filter' which is interposed

probably are identified with guanosine, adenosine and nucleosides Thus, by injury or death, any cell may exhibit a form of trephocytic relation to other cells

The supernumerary spermatozoa in polyspermy become trephocytes for the cells of the developing embryo[194] chiefly by supplying additional DNA and some RNA,[103, 106] and probably a significant amount of hyaluronidase[393] and other substances Thus the cells of the embryo are the recipients of the trephones released by death of the supernumerary germ cells in polyspermy and polyvitelliny, whereas the oocyte is the recipient of trephones supplied by the follicular cells of the ovary or by the nutritive egg cells

Trephones are substances which are usually synthesized or degradated, and then released into the circulation or, they may be stored and transported in cells to the site where they are released for use by other cells Carrel[14] holds that there are 'hypothetical substances' called "trephones" *which certain cells in the body produce for the use of other cells in forming* their protoplasm The action of trephones is not necessarily specific, as is shown by the fact that chick embryonic tissue may be cultured in media prepared from embryos of the mouse, guinea pig or rabbit[258] Since trephones are not species specific one may be reasonably certain that their chief function is to supply certain substances in a readily available form Trephocytes are probably widely distributed throughout the metazoa because they occur in many invertebrates,[606] in certain insects[910] and in most of the vertebrates[605]

Functions of trephocytes are sometimes overlooked because a cell may have more obvious characteristics which are indicative of a minor function The granules of mast cells contain AMP which are easily identified by many staining methods Granules of mast cells contain histamine as well as heparin or other forms of AMP The chief importance of mast cells in collagen formation is probably because they are a source of histamine which increases the permeability of capillaries and promotes leakage of proteins, enzymes and other substances into the tissue fluid

A second difficulty in establishing the function of certain trephocytes is that other cells may contain varying quantities of the same substance as the trephocyte *Recognition of changes in the number of storage cells or the* amount of material in them is relatively easy, compared with detecting the effects when minute quantities of that same substance are distributed ubiquitously

Another point to be considered is that storage of a substance in one kind of cell may result in emphasizing its function in synthesis of that substance to the exclusion of synthesis of the same substance by other cells The localization of synthesis of globulin following injections of fluorescein labeled antibody is taken to indicate that plasmacytes synthesize antibodies

by later investigators who have shown that both the nucleus and the nucleolus in eggs of the sea urchin (Arbacia) behave osmotically[416] It has been demonstrated by the freezing microincineration technique that the mineral substances in various cells bear a quantitative relation to each other For instance calcium and magnesium were generally high in the nucleus, cell membrane and in certain other definite locations, but the potassium content was high where the calcium and magnesium content was low[4] Indeed, Churney[16] believes that the immature eggs of Arbacia behave almost as a perfect osmometer and that those of a marine worm, Nereis, are nearly as efficient The presence of nucleic acids in the nucleolus as well as in the cytoplasm surrounding the nuclear membrane[148] suggests that RNA, DNA or their components in resting cells pass through the nuclear membrane by dialysis rather than by being entirely dependent upon dissolution of this membrane during the prophase stage to enable these substances to enter the cytoplasm or nucleus Danchakoff (1916) observed a "basiphilic chromatic substance in the cytoplasm of starfish eggs which she believed passes into the nucleus for the purpose of contributing to the growth of nuclear chromatin[18] Many investigators report the passage of nuclear chromatin into the cytoplasm

There appears to be a fairly definite DNA to RNA equilibrium in resting cells When there is an imbalance between DNA and cytoplasmic RNA something rather drastic will likely happen to the cell, such as the initiation of mitosis or necrosis The explanation is not so simple as an imbalance between DNA and RNA, in either structure for the presence of these two nucleic acids probably represents an interaction, if not the culmination of a series of actions of a number of enzymes coenzymes and catalysts (some of which might be anticatalysts) on various substrates To illustrate this point Caspersson[148] stresses the importance of the nucleus in protein synthesis, but Brachet[104] thinks that the cytoplasmic granules take precedence in importance in this process Haurowitz and Crampton[411] cite works which show that the content of peptidase in the cytoplasm of frog oocytes is increased as the yolk proteins are being formed whereas the nuclear content of peptidase remains low at this time Brachet[104] suggests another angle by stating that the distribution of sulfhydryl proteins and of cytoplasmic RNA is equal, whereas the nuclear sap although rich in —SH groups contains no nucleic acid

Nucleocytoplasmic Interrelationships

Various specialized normal nucleocytoplasmic relationships range from the binucleate sporulating cells of *Bacillus megatherium*, the macro- and micronuclei of certain protozoa and the vegetative pollen tube nucleus in pollen grains of Tradescantia[807] to the phenomenon known as chromatin

between the hepatic portal organs and systemic blood The reticuloendothelial cells in lymph nodes have similar phagocytic functions, but are bathed by peripheral lymph composed chiefly of tissue fluid

Often lymphocytes and plasmacytes form aggregates in normal tissues, and are more conspicuous than several other cells which do not usually form aggregates Also, eosinophils and neutrophils infiltrate tissues at an early stage of inflammation or of wound healing and may have a function *in the catabolism of components of necrotic cells in addition to the* phagocytic function of neutrophils

Phagocytosis of bacteria, India ink, other particulate matter, cells and cell debris, is an obvious function of certain phagocytes in the spleen and lymph nodes and is readily demonstrated However further information on the functions of lymphocytes and lymphoid organs in protein metabolism depended upon the use of several of the more recently devised biochemical and tracer methods Use of paper electrophoresis has provided information on changes in the plasma protein in many physiological and pathological conditions Use of radioactive carbon, iron nitrogen, phosphorus, sulfur and other elements has provided information necessary for a better understanding of the synthesizing and storage functions of eosinophils mast cells, lymphocytes and plasmacytes

The formation of variously differentiated cells from a single stem cell is a regulatory mechanism Some hematologists[7] believe that reticuloendothelial cells develop into histioblasts monocytes mast cells plasma cytes and tissue eosinophils Conditions conducive to protein synthesis may cause the increased formation of monocytes which have a very high content of RNA whereas excessive histamine and heparin in tissue fluid may stimulate the formation of mast cells Thus, undifferentiated mesenchymal cells may retain several substances in an immediate area in addition to forming monocytes which pass into the blood

INTRACELLULAR SUBSTANCES AND FORCES

Substances within cells are related to or are dependent upon nuclear and *cytoplasmic structures Intracellular substances include nucleic acids,* nucleotides[148] and their derivatives sulfhydryl proteins[104] free proteins enzymes vitamins salts lipids trace elements[1043] and other materials[416] Most of these substances are often in intimate interrelationship with organelles (mitochondria and Golgi apparatus), submicroscopic bodies or microsomes, and obscure substances Some are further complicated by varying degrees of polymeric[98] ionic[416] permeability or other relations within the cell or its medium It is evident therefore that osmotic pressure undoubtedly plays an important part in the exchange of autotrephones as was indicated by the early work of Hamburger (1902 to 1904) and verified

by later investigators who have shown that both the nucleus and the nucleolus in eggs of the sea urchin (Arbacia) behave osmotically[416] It has been demonstrated by the freezing microincineration technique that the mineral substances in various cells bear a quantitative relation to each other For instance, calcium and magnesium were generally high in the nucleus cell membrane and in certain other definite locations, but the potassium content was high where the calcium and magnesium content was low[24] Indeed Churney[16] believes that the immature eggs of Arbacia behave almost as a perfect osmometer and that those of a marine worm, Nereis, are nearly as efficient The presence of nucleic acids in the nucleolus as well as in the cytoplasm surrounding the nuclear membrane[148] suggests that RNA, DNA or their components, in resting cells pass through the nuclear membrane by dialysis rather than by being entirely dependent upon dissolution of this membrane during the prophase stage to enable these substances to enter the cytoplasm or nucleus Danchakoff (1916) observed a ' basiphilic chromatic substance in the cytoplasm of starfish eggs which she believed passes into the nucleus for the purpose of contributing to the growth of nuclear chromatin[18] Many investigators report the passage of nuclear chromatin into the cytoplasm

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elimination, or formation of chromidia, as designated by R Hertwig (1902) [110] These relationships involve intricate, although well organized but often obscure interchanges between the contents of the nucleus and the cytoplasm in the cell [103, 106, 167, 188, 44, 747, 9, 973] Outpocketings of the nuclear membrane permit passage of material from the nucleus into the cytoplasm,[338] and associated dense chromosomal material releases material into the cytosome where endoplasmic reticulum and cytoplasmic particles are formed [38] The fact that it is stated that the blebs of nuclear material are associated with heterochromatic chromosomal regions indicates that the material being extruded is chiefly RNA

Exchange of material between the nucleus and cytoplasm is usually by dialysis of submicroscopic particles or substances Diffusion of partly hydrolyzed substances which are not indicated by histochemical staining methods,[910] or dialysis during the interkinetic phase, has been considered the method for passage from the nucleus to the cytoplasm Schrader and Leuchtenberger[910] note that follicular cells of Hemiptera furnished nuclear and cytoplasmic material to the egg by extrusion through the nuclear membrane Another possibility may be extrusion of DNA through pores in the nuclear membrane, such as occurs in cells in an acid solution [7]

Enucleation or nuclear injury has been used extensively to determine nucleocytoplasmic interrelations As early as 1878 Claude Bernard recognized that the nucleus was essential for cellular function and suggests that it is ' the cell organ which is most essential to synthetic metabolism "[9, 8] Because of its recognized basic implications, publication of this statement was followed by a number of experiments with living, enucleated ova, protozoa and other cells that extended over a period of years[1, 3, 714, 9, 8, 110] and which supported if they did not actually prove, Bernard's idea Chambers[1, 3] summarizes the results of these experiments by stating that there is no recorded instance in which cell division has ever been experimentally induced or has occurred in the absence of an active nucleus or its active material except by the use of plasmolytic methods Apparently cleavage of enucleated eggs may be induced only in certain mature marine eggs in which a small part of the nucleus is visible and may be removed the major part having become invisible by diffusing throughout the cytoplasm Consequently the nucleus of these eggs which may cleave a time or two, has merely been seriously injured at most

Those who are interested in the interpretation of some of the nearly unbelievable variations of nucleocytoplasmic relationships should also consult the more recent works of Horstadius and collaborators on the development of enucleated blastomeres of sea urchin eggs [449, 617] Patterson and his colleagues and others described the development of the armadillo (*Tatusia novemcinctus*) the egg of which normally produces four embryos, and

Patterson also studied the polyembryonic gall forming wasps (Paracopido somopsis) in which a single egg is normally capable of producing dozens of embryos[787, 1033]

The intricate interchanges between nucleoplasm and cytoplasm appear to be affected by several factors, chief of which appears to be enzymes which may differ in kind or in other ways The concentration of only a few enzymes appears to be higher in the nucleus than in the cytoplasm of the cell However, certain other enzymes such as succinic dehydrogenase are absent in the nucleus although present in the cytoplasm [4]

Many vitamins serve with the enzymes as coenzymes or as cofactors in affecting some of the nucleocytoplasmic interrelations in cells Available information on the concentration of the vitamins and minerals in the nucleus is probably more accurate than that obtained for the cytoplasm, because the nuclei in these investigations were usually removed from the cells of the tissue by Behren's method or a modification of it and analyzed microbiologically Subsequently entire cells were analyzed and the cytoplasmic value was usually taken to be the difference between these two values Unfortunately, injury changes may occur, and the investigators commonly give only the nuclear to whole cell values As an example, Williams and co workers (1942) report that the nuclei in cardine muscle contain 2 to 4 times as much folic acid, nicotinic acid pantothenic acid riboflavin and thiamine as the whole cell, pyridoxine is about as equally concentrated in the cytoplasm as in the nucleus, and conversely, biotin and inositol were found to be more concentrated in the cytoplasm than in the nuclei [4]

It is a well established fact that, with a few possible exceptions such as a sea weed[416] the cytoplasm is quite unable to maintain its internal morphology or to form new chemical products in the absence of the nucleus, as is shown by Dounce (1952)[95] The results show that the cell dies without reproducing itself[135, 751, 110] and that the cytoplasm has, at least in most forms a determining influence on the behavior of the nucleus especially in ontogeny[110]

The significance of autotrephones in the physiological organization of certain cells is shown by the fact that removal of a portion of the cytoplasm without injuring or disturbing the nucleus in unsegmented eggs of such animals as nemertines ctenophores and molluscs caused developmental defects in embryos[110] It has also been shown by microdissection that the ameba will not divide if an intolerable amount of its cytoplasm has been removed However, after the organism has replaced its missing cytoplasm to near the normal nucleocytoplasmic ratio it renews its mitotic activity[24] Even a slight prick of the cytoplasm in one of the primary blastomeres in eggs of fresh water snails with a fine glass needle disturbed cleavage and

caused the death of the embryos within 3 days[190] However, the eggs of certain other animals are so "organized" that fragments may undergo varying degrees of development and, in some isolated blastomeres are 'totipotent' and may develop skeletal[806] or other parts, incomplete embryos or normal but dwarfed embryos[187, 193, 448, 714, 1107]

Chromatin Elimination

It is a well established fact that an appreciable amount of chromatin is cast out of the normal nucleus during the prophase stage of mitosis in a number of different cells especially auxocytes[18, 794, 1016, 110] which are identified in the cytoplasm as chromidia[447, 1102] or later, as volutin[9, 3] Bonnevie (1905) suggested that the process of casting chromatin out of the nucleus into the cytoplasm is not limited to the maturing ovum, but may also occur during mitosis of all somatic cells[110] This tenet is historically significant because it infers that trophochromatin (chiefly RNA) is needed and utilized in the process of mitosis wherever it occurs and that it is not limited to the formation of deutoplasm in the ripening egg cell More recent work shows that chromatin elimination, or extrusion of DNA from the nucleus into the cytoplasm may occur in any cell undergoing mitotic division and occurs in cells of animals which range from a frog fluke[794] to mammals[167] It appears in the gametocytes and continues during cleavage and development[1107] Ris and Kleinfeld[867] used the eggs of a lepidopter (Solenobia) and regarded the chromatin elimination as a means of eliminating superfluous ribonucleoprotein from the chromosomes In this connection it is interesting to note that Brachet[104] believes that cytoplasmic RNA serves as a reserve material for the synthesis of DNA by the chromosomes and for the synthesis of protein

The process of chromatin elimination from the nucleus with the formation of chromidia[1107] afforded the earliest visible support of the tenet that there is an intimate interdependence between the nucleus and the cytoplasm It is now generally recognized that there are obligatory relationships between the nucleus and the cytoplasm, that nucleic acids are increased in both the nucleus and cytoplasm during the resting stage and that immediately after mitosis the amounts of DNA in the nucleus and of the RNA in the cytoplasm are markedly reduced in the forming daughter cells[104, 416, 807]

Intracellular Protein Synthesis and Storage

An abundance of extracellular protein is essential for active intracellular protein synthesis Likewise, a proper relationship between extracellular and intracellular salts anions and cations, must be maintained as a prerequisite for efficient extra and intracellular protein metabolism before

intracellular synthesis and storage of proteins can be effected Several structures within cells, including the nucleus nucleolus and cytoplasmic inclusions, have a certain degree of "storage function for intracellular biochemical processes Also, free amino acids, enzymes and other substances occur in the cytoplasm The free intracellular components are probably primarily concerned with intracellular metabolism However, the storage organelles in trephocytes may have appreciable significance in the metabolism of other cells locally, or in supplying substances for body fluids, especially for intercellular fluid The significance of trephocytes should also be considered in relation to the possible functions of their organelles but in some trephocytes the organelles are depleted or insignificant in the mature cell

The cell organelles include cell inclusions organoids particles cellular particulares,[110] cytological constituents,[189] the microsomes, or small particles[167] and secretory granules[4] These organelles are present in the cytoplasm of most cells, especially secretory cells Other designations used for cytoplasmic inclusions which have not been adequately described but should be mentioned are ultrachondriome (possibly a phase of mitochondria) observed by Porter and Thompson (1947)[? 1] and particulate globules (a general term apparently intended to include mitochondria, granules and others)

The types of cell organelles mentioned here are the Golgi apparatus (G bodies) mitochondria (chondriomes)[110] and microsomes (cytoplasmic particles 50 to 200 μ in diameter)[108] All three of these organelles occur in the cytoplasm of most animal cells from protozoa to man and in the cells of many plants Cell organelles are generally pleomorphic structures which are capable of division during mitosis of the host cell and, like chromosomes of reconstructing the entire organoid complex from the reduced amount remaining in each of the daughter cells[110]

Golgi apparatus and mitochondria are readily soluble in lipoid solvents, are argentophilic (especially the Golgi apparatus)[1119] and blacken when treated with osmic acid This indicates that both structures contain lipoids While the Golgi apparatus is in the granular or filiform stage, it is very difficult to distinguish it from mitochondria in a similar stage

It appears that the chief importance of cell organelles stems from their content of, or effect on active enzymic substances These substances are essential to the metabolism of the cell and include enzymes which are usually thermolabile colloidal protein substances[434] coenzymes which are thermostabile dializable non protein substances'[434], inorganic catalysts (*e g* calcium cobalt magnesium) and substrates including nucleotides and their derivatives Most of the enzymic reactions require a very delicate arrangement and balance of the substances and conditions that

*form the medium in which they function A very slight qualitative or quan*titative deviation in this medium may readily result in alteration, reversal or complete failure of the function of certain of the enzymes

There is a tendency to regard microsomes mitochondria and the Golgi apparatus as physiologically and chemically similar, but morphologically different structures[1119] Worley and Spater[1110] found evidence suggesting that 'Golgi bodies and mitochondria may at times form complexes between themselves or that mitochondrial material may become incorporated into the Golgi spheres" They point out that Adamstone (1950), Parat (1928) and Oliver (1948) have contributed information which supports their observations and that Ralph (1946) states that the granules of eosinophils 'appear to be composed of a nonlipid substrate 'saturated with acetone soluble lipids and a thin surface film of phospholipid " which suggests "that these lipids represent the Golgi mitochondria complex [708] Worley and Spater[1110] cite work of other investigators which indicates further relations between *Golgi* apparatus *mitochondria and mast cell granules, for the* techniques used to show Golgi material and mitochondria demonstrated only mast granules

Eichenberger[7 6] found by electron microscopy that about 10 days after the mitochondria had been depleted in certain cells of the kidney by intraperitoneally injecting mice with egg white the organelles redeveloped from microsomes apparently by aggregation Worley[1119] suggests that the idea that the Golgi elements arise *de novo* may be explained by the aggregation of particles of submicroscopic size Palade [7-] observed that the mitochondria in the cells of all tissues examined had a membrane 7 to 8 mμ thick which effectively separated them from the general cytoplasm and supported typically arranged christae mitochondrales His work supports the findings that some, if not all mitochondria contain phospholipids, proteins and a certain amount of RNA They are also associated with many enzymes some of which apparently 'are built up of a great number of enzymatic units " which are capable of a surprisingly wide range of oxidative activities coupled with the phosphorylation of adenine nucleotides,' and a capacity for certain synthetic processes All of these indicate that mitochondria play an important part in the cell s physiology and apparently are the chief source of the system of the supply of energy for the cell The very distinctly differentiated Golgi apparatus and mitochondria in the cells of sarcoma 180 reminded Worley and Spater[1110] 'of the spherical Golgi bodies as representing aggregations of lipoidal substances in areas of diffuse lipoid and of the mitochondria as somewhat similar aggregations of material within the ribonucleic acid containing cytoplasm Electron microscopy shows that the Golgi substance has both osmophilic and osmo

phobic components[1] to contain phospholipid and to be a 'dynamic constituent of the cell[78]

Typical examples of Golgi bodies and of mitochondria are easily identified but differentiation of these two cell organelles may be difficult or even impossible in sectioned material Some investigators believe that for vital staining methylene blue is specific for Golgi bodies and Janus green B for mitochondria, but others have had little success in staining Golgi bodies at all, and mitochondria were stained with difficulty except postvitally[1119, 110]

THE NUCLEOLUS

The true nucleolus consists chiefly of RNA *It is believed to function in* protein synthesis and storage within the nucleus, and to be related to the various aspects of cellular protein synthesis Many investigators have reported the extrusion of nucleolar substance into the cytoplasm as a common occurrence whereas in oogenesis this process is believed to be concerned *with the formation of protein yolk*[1119]

A number of papers published in the 1880 s and 1890 s contained statements indicating that nucleoli are centers for the elaboration or storage of chromatin kinetoplasm,' 'plastin" nucleic acid and other substances destined to play a definite part in nuclear functions[110] Some of these investigators believed that there is a very significant interrelationship between the nucleolus and the chromosomes (heterochromatin, or RNA and euchromatin or DNA) and between the nucleoplasm and the cytoplasm in living cells from plants and Protozoa to mammals[18, 98, 110]

The term nucleolus was used in a paper by Bowman as early as 1840[337] but its structure and functions remained a mystery until the use of RNAase, DNAase and various selective staining methods (including Feulgen s) became available Earlier investigators believed that the nucleolus probably held reserve material of the nucleus" that 'it was an accumulation of nutritious material for the formation of chromatin and some linked it with yolk formation[337, 1119] Strassburger believed that since the nucleolus is structureless it must play a passive part in cell physiology, and the idea that the nucleoli merely float freely in the nuclear sap" was common[337]

The nucleolus characteristically disappears *from the nucleus as the nu*clear membrane disappears during prophase and again becomes conspicuous during the resting or interkinetic phase of the mitotic cycle Thus, the nucleolus is sometimes considered to be an interkinetic cell organelle which forms its ribonucleoprotein during the telophase while the chromosomes also are reforming[913] Brachet[104] states that Hyden and Caspersson believe

form the medium in which they function A very slight qualitative or quantitative deviation in this medium may readily result in alteration, reversal or complete failure of the function of certain of the enzymes

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During the intermitotic phase this type of nucleolus may become intensely basophilic. Wilson[110] points out that the same nucleolus may be oxyphilic at one time and basophilic at another, and, that oxyphilic nucleoli are more readily digested by pepsin than are basophilic nucleoli. He wisely concludes that the changes in staining reaction of the nucleolus are due to changes in the chemical constituents or in their proportions, for a characteristic of true nucleoli is a high RNA content.[148, 2, 411]

The chromosome nucleolus or karyosome is composed chiefly of chromatin material and commonly persists as a strongly basophilic, spheroidal mass within the interkinetic nucleus. Thus, it characteristically contains a high proportion of DNA.[143] The amphinucleolus has been called a cytological alibi, for it is essentially a double nucleolus, part of which is composed of true nucleolar (high RNA) substance, and part of chromosome nucleolar (high DNA) substance. Wilson[110] states that this type may give rise to a true nucleolus and a chromosome nucleolus within a single nucleus. Schultz and co-workers,[913] citing the work of Baur on chironomid flies, point out wide variations in staining affinity of the nuclei of closely related species. Others have observed similar differences in individuals of the same species.[110]

There is abundant proof of the occurrence of amphinucleoli in which one area is basophilic and another is oxyphilic, of multiple nucleoli (the number of which is thought to be correlated with the number of genomes in certain plants)[9, 5] in which all or approximately half of the two to dozens of nucleoli may be basophilic and others oxyphilic, and of a basophilic nucleolus accompanied by acidophilic chromosomes.[110] These mixed staining reactions pose a problem in cell metabolism which is incompletely understood. Cytologists have been fairly successful in correlating the basichromatic and oxychromatic changes with mitotic phases. It is now generally acknowledged that while the nucleus is without a membrane during the later part of the prophase, the true nucleolus disappears and that it undergoes dissolution and contributes an appreciable amount of its substance, chiefly pentose nucleic acid (including RNA) to the cytoplasm.[104, 416, 511, 973, 1016, 110] The typical nucleolus contains RNA-rich heterochromatin and is now believed to regulate the synthesis of cytoplasmic proteins.[148, 411]

X irradiation (10 000 r.) of neuroblasts in grasshoppers during the telophase caused the characteristically dual nucleoli to subdivide into several spherical masses, but irradiation apparently had no visible effect on the nuclei when administered during any other stage of mitosis.[143] After a lapse of 25 to 35 minutes, ultraviolet irradiation (2250 to 3130 Å.) caused the nucleolus of grasshopper neuroblasts to fragment and form about 10 highly refractile spherules *in vitro*. These and other reactions suggest that the ultraviolet rays affect both the nucleic acid and the protein in the

that nucleoproteins aggregate near or pass through the nuclear membrane, but "the reverse idea, that the cytoplasmic nucleoproteins diffuse through the nuclear membrane and converge in the nucleolus cannot be completely excluded"

Caspersson thinks that euchromatin controls the synthesis of the complex, specific proteins of the genes and that heterochromatin regulates the synthesis of histones which accumulate in the nucleolus and later diffuse into the cytoplasm[104] Monné postulates that the euchromatin synthesizes the nuclear sap and by its intervention regulates catabolism within the cell, and that the heterochromatin controls anabolism by insuring the production of RNA within the cell[104] Other investigators believe that only heterochromatin contains both RNA and DNA[104]

It has been pointed out that in certain ways "the study of the nucleolus occupies a key position in the analysis of the interrelations of the nucleus and the cytoplasm"[913] Brachet[104] believes that the "nucleolus is composed of histones associated with ribonucleic acid in varying proportions" Schultz and co workers[913] point out that the nucleolus resembles cytoplasmic structures in containing RNA but that "like other genetic effects, it is related to a definite locus in the chromosomes" Brachet[104] believes that heterochromatin in the chromosomes gives rise to the nucleoli to which they are attached He also states that the nuclei of males contain more RNA but less of the histones than the nuclei of females

Schultz and co workers[913] point out that the nucleolus in *Drosophila melanogaster* has been shown to be associated with the heterochromatin of the X and the Y chromosomes and that the heterochromatic regions in the nucleolus and the cytoplasm produce the ribonucleic acid compounds These investigators think that the development of the nucleolus follows a pattern of gene function the ribonucleoprotein is formed at telophase and in endomitotic nuclei grows parallel with the growth of the chromosomes"

Living nuclei of certain plants and Protozoa appear to be composed of highly viscid jelly when examined by microdissection When the nuclear membrane of a living somatic animal cell is torn the immediate effect of the injury is a dissolution of the nucleolus"[1 3] In general the works of Beams[5] and Beams and King[6] in which ultracentrifugal methods were used indicate that the formed nucleolus has the highest specific gravity of any of the nuclear substances

Cytologists recognize at least three loosely defined types of nucleoli by their staining reactions The true nucleolus (plasmosome of Ogata 1883) was identified as staining with plasma or acidic dyes by Ogata in 1883[110] It is known that this oxyphilic reaction is not a dependable criterion because it is fairly well correlated with the prophase stage of the nucleus

phosphatases, RNAase and about half of all the esterase and RNA in liver cells have been found in the microsomes in embryonic tissues and in adult tissues and tumors

Differential centrifugation of liver, tumor or other homogenates is the usual procedure for obtaining microsomes Bradfield[108] indicates that the enzymic activities usually ascribed to these bodies (they are not usually ranked as high as organoids) when centrifuged at 18,000 g remain in the supernatant 1 to 2 hours The centrifugate may either be examined with the electron microscope or tested for enzyme activity to determine whether it contains microsomes This method probably never yields anything near a pure suspension of microsomes because it would be expected to include fragments of cells mitochondria Golgi apparatus and possibly other materials Bradfield[108] sounds a note of warning on the method of differential centrifugation by stating that it is not known whether the substances in the supernatant "are freely dissolved in the protoplasm or are eluted from the particles during separation This question of yield and purity of the material thus obtained (and of its evaluation) has been discussed at length [348]

nucleoli [143] Other writers present results which indicate that irradiation affects the euchromatin in the nucleolus, as is suggested by the vacuolation of both the nucleoli and chromatin particles [89]

Dearing [3] finds that somatic nucleoli (usually two in an epithelial cell) of the urodele, *Amblystoma tigrinum*, are portions of distinctive chromasomes He also states that when the interkinetic nucleolus is stained by the Feulgen method the peripheral granules are distinct, but "the center never takes the reaction" This characteristic plus the fact that, although the entire nucleolus stains deeply with Heidenhain's hematoxylin, the peripheral granules retain the dye more tenaciously during excessive destaining with iron alum, indicate that these nucleoli are chemically related to the amphinucleoli of earlier investigators,[30] [148] [4] and, that the center of the nucleolus is composed chiefly of RNA which is enveloped by a relatively thick layer of DNA granules which form 'nucleolar associated chromatin" Sharp[9] [8] suggests that the presence of a stainable sulphuric ester of a polysaccharide is responsible for the affinity of part of the nucleolus for iron hematoxylin When the results of these staining reactions are associated with Dearing's [9] statement that the nucleolus becomes chromophobic and disappears by gradual diminution rather than by fragmentation during the middle prophase, the autotrephogenic nature of these somatic nucleoli and their interrelations with nuclear chromatin become obvious

MICROSOMES AND SECRETORY GRANULES

Microsomes are another intracytoplasmic source of RNA The microsomes or small particles [167] are nearly submicroscopic granules which range from 50 to 200 mμ in diameter [109] The electron microscope shows that small microsomes have a limiting membrane and are small (10 to 25 mμ) intermediate (20 to 80 mμ) and large (70 to 150 mμ) [9] [0]

Cells of the submaxillary gland and pancreas contain a high proportion of microsomes compared with cytoplasmic particulate fractions in the adrenal thymus and in some tumors [78] Approximately 80 per cent of all the RNA in the cells of the submaxillary gland is associated with the cytoplasmic microsomes and non sedimentable proteins [678] Claude (1943) suggested that microsomes and secretory granules may have a common origin and that either may contribute to the constitution of the other [578] Microsomes and secretory granules are composed of phospholipids and ribose nucleoproteins However microsomes have 40 to 45 per cent lipid whereas secretory granules have about half as much (20 to 24 per cent) lipid Woolley determined that the amounts of inositol a B vitamin are proportional to the amounts of lipids in both microsomes and secretory granules [167]

Microsomes of normal animals contain a variety of enzymes certain substrates and other essential substances Catalase, dipeptidase, various

✓ administration of cortisone over a long period[06] However, RNA in hepatic cells was significantly increased in pregnant rats during the last week of gestation[138]

The amount of cytoplasmic RNA in basophils of the anterior pituitary gland also fluctuates Cytoplasmic basophilia in basophils of the anterior pituitary is attributed to the presence of RNA because basophilia is decreased following treatment with ribonuclease (RNAase)[99] Structural and physiological changes in pituitary basophils have been correlated with reproduction and with fluctuations of metabolism in a hibernating bat *Myotis lucifugus*[919] Basophilic cells in the pituitary increase in thyroxin deficiency and after administration of thiourea[815] Prolonged administration of adrenocorticotrophic hormone (ACTH) decreased basophilia in the anterior pituitary There was also loss of weight of the hypophysis and depression of its functions but the acidophiles were not altered[6] Estrogens caused degranulation of basophilia in this gland[1113] Extracts of basophilic tissue from the hypophysis have greater concentrations of thyrotropic adrenotropic and gonadotropic hormones than the more predominantly acidophilic and chromophobic portion of the anterior pituitary of cattle[9 1]

The high rate of protein synthesis in various cells of the anterior pituitary suggests that these cells may also be provided with satellites or trephocytes Shanklin[9 7] found that areas of lymphocytes and lymphoid tissue occurred in 43 of the 100 human pituitaries examined and were usually present in the pars intermedia The positive cases occurred in 35 7 per cent female and 71 7 per cent male pituitaries but were not present in 26 specimens from individuals less than 35 years old

Disintegration of all cells releases amino acids However this cytolysis is not quantitatively important in normal tissues nor are the cell components in a 'packaged' transportable form—in contrast with the disintegration of lymphocytes which may have been transported intact from a distance or may have transformed into plasmacytes before disintegrating[534] Amino acids from histones in the nucleus of erythroblasts for example may be used in synthesis of globin in the cytoplasm of the same cell or the extruded or lysed nuclei of mammalian erythroblasts might provide amino acids for protein synthesis in other hematopoietic cells such as the formation of hemoglobin by hemocytoblasts or gamma globulin in the cytoplasm of plasmacytes Histones are probably hydrolyzed in the plasma to amino acids which subsequently are combined into various proteins such as albumin fibrinogen globulins globin lactoglobulin casein and other proteins because there are differences in the arginine content of hepatic cells and erythrocytes[977]

The substances stored by lymphocytes and plasmacytes are in a readily

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Lymphocytes and Plasmacytes

The intracellular retention of nucleoproteins is probably more important physiologically and chemically than is the intracellular storage of glycogen in hepatic cells and of fat in adipose cells. Lymphocytes and plasmacytes are the cells whose primary purpose appears to be the synthesis and storage of nucleoproteins for use by other cells. Nucleoproteins are sources of amino acid, purines and pyrimidines and of ribose and deoxyribose. Furthermore, nucleic acids may furnish mononucleotides for the formation of coenzymes or other products which are essential for protein synthesis. Ehrlich and Seifter[5] postulate that lysis of lymphocytes and plasmacytes may furnish nucleic acids and their split products and that lymphocytolysis furnishes not only building stones but also energy for synthetic processes.

The plasma proteins and lymphoid tissues are the most obvious sources of these stored proteins. However, less obvious forms of nucleoprotein storage occur in organelles within the nucleus and cytoplasm of most cells. A reservoir of protein has been considered by Whipple and colleagues to be present in certain cells as a fluctuating and integral cell protein.[140] That intracytoplasmic proteins can serve as a reserve of protein in certain tissues has been strongly indicated. For instance, exhaustive muscular exercise decreases protein and RNA in the cytoplasm of the affected motor cells.[989]

Storage of RNA occurs in plants as well as in animals. Cotyledons of plants function as storage organs for ribonucleic acid (RNA), which decreases very rapidly during germination. However, it is not known whether the entire molecule of RNA or only its components are transported.[7] Free amino acids are believed to be unimportant as storage products since they are not depleted by starvation.[160] Some of the RNA in the cytoplasm of hepatic cells may be considered to be in the class of dispensable proteins, and includes proteins utilized during stress.[9] Statkewitch reported that the liver is the organ most affected by inanition and is followed in sequence by the kidneys, parotid and submaxillary glands and the pancreas.[690] RNA was almost entirely depleted in central hepatic cells of mice that were subjected to 4 to 8 days of starvation[1098] and was also decreased in hamsters which had been starved[535]—and in those receiving

when it is needed and promotion of cell proliferation" A trephocytic function for leukocytes was specifically applied to cancer by Virchow,[1060] who suggested an analogy between the development of the cancer cells and the ovum In 1901, Levin[533] stated that proliferation of cells may be stimulated by an external agent

The specific function of lymphocytes (including thymocytes) as storage cells for nucleoprotein was considered by Jolly[14] and Dustin,[7 9 9 60 61 6] who believed that (1) the thymus has a function in nuclear regulation, (2) the formation and lysis of lymphocytes regulate the available nucleoproteins, (3) a reserve of nucleoproteins is necessary for all active cell division, and (4) lymphocytes and lymphoid organs have a function in cytoregulation in adult animals

The investigations of Watjen [9] were verified and amplified by Dustin during the years 1907 to about 1921 [8] Bristol[111] was a practical exponent of the importance of lymphocytes in growth processes He states that in his opinion the chief physiological function of lymphocytes, which are composed chiefly of nuclear material, is 'the carriage, in the form of nuclear enzymes, of energy that is required by normal living body cells in the process of growth and multiplication ' He also believes that 'under abnormal conditions and in concentrated numbers these lymphocytes may give additional aid and energy to the normal body cells surrounding areas of injury and inflammation

Not only did Bristol consider the energy relations of lymphocytes he also observed the similarity of the nucleus of lymphocytes to embryonic cells and recognized the aggregation of lymphocytes at sites of injury as a method of aiding tissue repair During the period from 1919 to 1950 considerations of the growth promoting functions of lymphocytes were rare and in some cases actually suppressed because of the predominant acceptance of the idea that lymphocytes are factors in resistance to bacterial infection and cancer growth, and are a source of antibodies A review of Carrel's[144 145] work in tissue culture shows that the lymphocytes have growth promoting properties Carrel and Ebeling[146] found that the addition of lymphocytes or of leukocytic extracts was followed by mitotic proliferation of epithelial cells and fibroblasts *in vitro* Lymphocytes were also considered by Jordan and Speidel[498] to be embryonic relics which stimulated the growth of the limbs in tadpoles treated with thyroid Kelsall [15] considered some of the correlations previously mentioned by Bristol and included material from research on the mammary tumor incitor and new data in endocrinology

Lymphoid tissue is advantageously situated between certain free body fluids and the vascular systems The spleen stores nucleoprotein within the blood vascular system between peripheral arterial and portal venous

expendable form In many cells there is a less expendable form of intra cytoplasmic protein which fluctuates very little in quantity under normal physiological conditions, but is depleted during starvation or other forms of dietary restriction Laird and Barton[570] found that starvation causes a reduction in nuclear RNA and protein but to a lesser extent than in other cell fractions, and that re feeding caused a rapid return to normal levels in protein and RNA of the nuclear fraction

LYMPHOCYTES AS TREPHOCYTES

As early as 1715, Dionysius believed that the fluid secreted by the thymus served as nutritive material for the newborn, and this idea was supported, in treatises on the lymph vessels of the thymus, by Codwper, Verheyen (1706), Haler (1748) Mascagnius (1787), Lucae (1811), Becher (1826), and Haug tead (1832) [9 6] In 1873, His first called at tention to the presence of leukocytes in the vertebrate ovary [604] Dustin [58] gives credit to Carrel and Ebeling for having solidly established the fact that leukocytes are the bearers of trephones' essential for the main tenance and growth of tissue cultures Dustin [9] states that the function of thymocytes is to store or to liberate nucleoproteins but this idea was not developed to determine the basic function of lymphocytes Although Mendel and Saiki recognized *d* ribose as a nucleic acid component and a product of developmental synthesis in animal tissues as early as 1908 [399] a quarter of a century passed before methods for evaluating the sig nificance of nucleic acids were available Thus, investigations to de termine the basic function of lymphocytes as trephocytes have been de layed or even suppressed at times chiefly because of the predominant idea that lymphocytes are defensive cells The fact that very few of the many diversified chemical relations of nucleic acids and their mononucleotides were seriously considered until comparatively recent years has impeded progress in this field The functions of nucleic acids and their mononucleo tides in synthesizing essential proteins in phosphorylation and as a source of radicles for coenzymes and adenosine triphosphate (ATP) are so diversi fied that the common and basic function of lymphocytes and plasmacytes in the synthesis storage and/or transportation of deoxyribonucleic acid (DNA) and RNA have been obscured The multiple functions of the nucleic acids parallel the problem of lymphocytes Drinker and Yoffey [1] state 'The lymphocyte seems to be almost ubiquitous and to be involved in the response to such a variety of pathological conditions that it is dif ficult to conceive of any function common to all these lymphocyte reac tions' Carrel[145] suggested in 1924 that our experiments indicate that lymphocytes remain through life as storage of embryonic growth promot ing substances as trephones which may cause a resumption of cell activity

are only two of many differentiated cells which do not divide *in vivo* Small lymphocytes and plasmacytes contain nucleic acids histone RNA and other proteins which are essential for cell division Mitosis of large lymphocytes usually occurs in the germinal centers of lymphoid tissues where these cells are the source of small lymphocytes Synthesis of DNA is believed to be limited to the interphase of mitosis[1015] Therefore, in lymphoid tissues, the large lymphocyte is the cell form that synthesizes the nucleoproteins which are stored and transported in the small lymphocytes

The motility and mobility of small lymphocytes enable these cells to transport a packaged, nucleoprotein unit intracellularly throughout the body This intracellular transportation is a means by which nucleoproteins can be concentrated when and where they are needed It is very efficient because small lymphocytes are numerous in the blood, lymph and tissue fluid and are capable of penetrating most soft tissues This transportation is facilitated because small lymphocytes are about the same size as the erythrocytes in most species of mammals [18, 580] For example, small lymphocytes of man are about 6 to 8 μ in diameter and erythrocytes average 7 74 μ [661, 668] Mature plasmacytes which are approximately twice the size of small lymphocytes do not usually occur in normal blood, but retain the nucleoproteins in the area where they are formed [34]

A characteristic of small lymphocytes is the predominance of the volume of the nucleus over that of the cytoplasm [78, 558] or the high nucleocytoplasmic ratio (Kern/Plasma ratio of R Hertwig) However, the nucleus of small lymphocytes has about the same amount of DNA as in most other cells, such as motor neurons,[899] but it contains few conspicuous cytoplasmic organelles[273] Nuclei of lymphocytes (calf thymocytes) after being denuded of cytoplasm, have been found actively to incorporate labeled amino acids into their proteins in a sucrose medium Apparently this incorporation of amino acids by the denuded nucleus of the lymphocytes is controlled primarily by the DNA within the nucleus Removal of the DNA from the nucleus virtually prevented the uptake of amino acids and the synthesis of protein by nuclei of lymphocytes This function of DNA can be assumed by partly degraded RNA or by certain other DNA (but not by pyrimidine) by purine bases and certain dinucleotides or mononibonucleotides[4]

Small lymphocytes may release RNA into intercellular fluids, since these cells have a thin shell of cytoplasm which has a high concentration of RNA as indicated by treatment with RNAase The RNA is released from intact lymphocytes by several means such as cytoplasmic budding, cytolysis (figs 4-6) and probably by dialysis[534] The transformation of lymphocytes into plasmacytes may be due to the failure at times of the

blood The lymphocytes and plasmacytes in the spleen function as an intracellular form of protein storage in which synthesis and release appear to be maintained in balance with the level of protein in the plasma which also serves as extracellular fluid for these cells in the spleen[534] Lymph nodes represent storage of nucleoproteins between tissue fluid and blood Intestinal lymphoid tissue functions as a nucleoprotein storage organ between the luminal fluid (chyle) of the intestine and a direct blood vascular connection (the hepatic portal vein) with the liver, in addition to the lymphatic drainage[534]

Another relation of extracellular fluid to protein storage is that differences in the amount of lymph in various lymphoid organs determine the degree of sensitivity of these structures to lymphopenic substances For instance, injection of cortisone into rabbits,[306] hamsters and mice[35] cause lysis of lymphocytes in the thymus earlier and to a greater extent, and also in response to smaller dosage than in lymph nodes The thymus has very few true lymph sinuses and less contained lymph than typical lymph nodes Thereby the decreased amount and flow of lymph results in a higher concentration of blood borne cortisone in the thymus than in the lymph nodes of the same animal

Incidentally, this unusual ability to concentrate administered substances is probably related to the observed greater sensitivity of the thymus than of lymph nodes to the administration of arsenical[60-64] and other lymphocytotoxic agents[534]

The relations of small lymphocytes and plasmacytes to protein synthesis varies in different tissues and organs In the gastrointestinal tract small lymphocytes and plasmacytes have nearly uniform functions in protein storage, therefore slight quantitative variations in them are not usually obvious However many authors have mentioned that they observed an increase in small lymphocytes and plasmacytes in the experimental production of gastric papillomas Small lymphocytes can supply 17 individual trephones for use by other cells or any combination of two or more of these trephones There is possible therefore a grand total (as shown by the formula $2^{17} - 1$) of 131 071 ways by which small lymphocytes may furnish trephones[534] This cell stores adenine phosphoric acid D 2 deoxyribose guanine cytosine thymine and adenosine monophosphate from DNA and the 10 amino acids reported to be present in thymus histone[41, 534]

Trephocytic Characteristics of Lymphocytes

There are a number of characteristics of the small lymphocyte which enable it to store transport and release nucleoprotein for use by other cells Absence of mitosis is a characteristic of most cells whose primary function is the storage of protein The small lymphocytes and plasmacytes

on a lymph node was reported by Florey (1928) to double the number of lymphocytes in the efferent lymphatics[893] The lymphocytic turnover has been considered to remain about constant because the number of lymphocytes in peripheral lymph and blood remains fairly constant[87–91, 94, 305, 11, 9]

Attempts to measure the lifespan of lymphocytes by incorporating radioactive phosphorus in the DNA have failed to give conclusive results Ottesen (1948) found that radioactive phosphorus injected into chickens was incorporated in the DNA in white blood cells[11, 7] Fichtelius (1951) by elaborating Ottesen s method, showed that following injection of P^{32} the appearance of radioactive lymphocytes in the blood reached two peaks of persistence, one about the 4th day subsequent to the injection and the other about the 15th day[11, 7] Later (1953), he showed that, by bleeding the P^{32} injected animal the latent period of the second peak could be reduced from 15 to as short a time as 4 days[11, 7]

Coassin and Kline[1] found, by the use of radioactive phosphorus that the life span of lymphocytes in the blood and lymph nodes is about 2 weeks in the rabbit but that the lymphocytes in thoracic duct lymph of non isotope treated rabbits are replaced twice daily (about every 12 hours) In their attempts to reconcile these disparate latent periods these investigators point out that annulation of the thoracic duct may have induced "an abnormal discharge of lymphocytes from the nodes" resulting in a 12 hour turnover whereas it is impractical to determine by the isotopic method how many generations of lymphocytes the DNA phosphorus had passed through previously

Coassin and Kline also found that within 2 5 hours after the intraperitoneal injection of 250 μc of inorganic P^{32} into rabbits lymph in the thoracic duct contained about 5750 P^{32} active lymphocytes and the lymph nodes about 8050 The number of lymphocytes reached its maximum in the duct (15 000) and in the lymph nodes (8400) at the 5th hour No P^{32} active lymphocytes were observed in heart blood during the 168 hours of this experiment These investigators conclude that the 15 day value subsequent to intraperitoneal injection of P^{32} represents a minimum possible life span of the lymphocyte these cells undoubtedly persist for an unknown additional length of time after leaving the blood stream as shown by the isotope method, whereas 12 hours is the minimum lifespan shown by the collection data for lymphocytes in the blood

Cyclic changes in germinal centers of lymph nodes have been well established even though the significance is not well understood[183, 1, 316, 1086, 11, 7] Flemming (1885) used the name 'germinal center to designate the delimited area of active proliferation in the postnatal lymphoid nodule[1127] Several other investigators found that the germinal centers hyper

lymphocytes to "excrete" sufficient quantities of RNA and other protein into the extracellular fluid, as well as to an increased synthesis of cyto plasm Figure 1 shows that RNA in the cytoplasm of small lymphocytes occurs as a very dense, compact mass in air dried blood smears stained with methylene blue at pH 4 9 by Haenel s[385] method It is not always possible to see the individual RNA particles because they are abundant and clumped together RNA positive particles in small lymphocytes are *about the same size as the abundant microsomes in the cytoplasm of hepatic* cells, as demonstrated in imprints of hepatic tissue stained by methylene blue at pH 4 9[534]

LIFE SPAN OF LYMPHOCYTES

A most significant quantitative point in considering the importance of lymphocytes in the synthesis, storage and transportation of nucleo proteins is the rapidity and extent of turnover and the life span of these cells[534] Several types of experimental studies have demonstrated that there is a rapid disappearance and replacement of lymphocytes in normal animals It has been estimated that some 200 million lymphocytes pass out of the thoracic duct into the blood stream every hour in the dog,[57 1] and that more lymphocytes enter the blood stream than are present in the blood at any one time[134 51 94 30 11 7] Jordan (1939) estimates that lymphocytes disappear in man at the approximate rate of 25 billion per day[134] which presupposes an equally fast replacement of the lost cells

Estimations of the life span of lymphocytes vary from a few hours to several days The average duration of lymphocytes in the blood was de termined as not exceeding 4 hours[11 6] This value was determined by Bunt ing and Huston (1931), and Yoffey (1933) who calculated the number of lymphocytes that pass daily from the thoracic duct into the blood stream in relation to the number normally found in circulating blood[30] Drinker and Yoffey[51] cite results which indicate that in dogs the blood lymphocytes have an average turnover of at least 3 5 times per day Yoffey and Courtice[11 7] give results of calculations indicating that the lympho *cytes may be replaced at least 3 71 times per day in the dog* McCutcheon[663] thinks that lymphocytes are probably renewed about every 8 hours Haden (1940) estimates that 24 hours is about the life of a lymphocyte and as sumes that the daily production is sufficient to replace this loss[134] How ever, lymphocytes observed through modified Clark Sandison windows in the ear of rabbits are reported to have lived at least 26 days[11 6]

Various approaches and experiments have been devised to obtain lon gevity values and other information on lymphocytes in the blood stream The known methods of investigating lymphocytic turnover are subject to considerable, usually unavoidable, error For example a slight pressure

of potassium which is essential for growth and regeneration [79, 1141] Increased renal loss of potassium occurs following the administration of ACTH,[79] but the extent to which this is due to lysis of lymphocytes, which also follows the administration of ACTH or cortisone [96] has not been determined

The death of all cells including erythrocytes, releases potassium [79] However, the significance of potassium in small lymphocytes is that lysis of this cell often occurs at the site of delimited growth such as wound healing In addition, iron which is stored in the spleen, liver, bone marrow and teeth of vertebrates,[134] is present in chromatin of nuclei in animal and plant cells, and is also locally concentrated from the disintegration of nuclei [134]

NITROGEN RETENTION

The intracellular storage of proteins in small lymphocytes and plasma cytes is regulated by certain hormones A shift of proteins from extra cellular fluids to an intracellular storage in lymphocytes might increase nitrogen retention, whereas a shift of protein from intracellular storage to extracellular fluid forms could be an early step in increasing protein catabolism Lymphocytes rapidly disintegrate and lymphoid tissues are depleted in starvation or as a result of protein low diet or of cortisone administration However lymphocytes and lymphoid tissues increase in hyperglobinemia and in other conditions of increased nitrogen retention

Several hormones control the amounts and ratios of the plasma proteins After hypophysectomy, serum globulin increases and serum albumin decreases Thyroidectomy, however, increases serum globulin, but does not affect serum albumin [600] Growth hormone and adrenocorticoids are antagonistic in many cases [836] Reid[836] states that the growth hormone and adrenocorticoids are not mutually antagonistic in carbohydrate metabolism However, the level of nucleic acid is increased by growth hormone and decreased by adrenocorticoids but dietary factors such as pyridoxine are also essential to increased nitrogen retention Lawrence and MacVicar [86] state that it is not known whether the increased protein excretion following the administration of cortisone is due to increased catabolism or to an antianabolic effect Synthesis and storage of protein in lymphocytes can explain both effects for cortisone causes lysis of lymphocytes and decreases lymphoid tissue and, in turn, it deters intracellular protein storage by decreasing mitosis

Lysis of lymphocytes provides a readily expendable source of nucleoproteins Lymphocytolysis occurs before the cytoplasmic proteins in hepatic cells decrease Protein depletion produced by low protein diet, caloric

trophied following the injection of foreign protein (bacterial antigens, broth) and were regarded as "reaction centers",[11 7] while others attributed the large size of lymphocytes in germinal centers to an increased level of available nucleic acid and its increased metabolism[1080 11 7] Thus, the finding that inanition decreases, and refeeding increases mitosis in the germinal centers in mice[316] is to be expected

Coassin and Kline[17] show that the incorporation of intraperitoneally injected P^3 into the DNA of lymphocytes was far more active at all intervals (ranging from 2 5 to 168 hours) in the lymph nodes than in lymph from the thoracic duct in rabbits Nevertheless, although they apparently agree with the tenet that P^3 is incorporated into DNA "only during the formation of a cell" and that the P^3 is released by the death of the cell, they do not think that re utilization of the isotope tagged protein by other cells would likely result in appreciable error in the values of the lifespan of lymphocytes obtained by the isotopic methods

The "daily replacement factor (D R F)" for lymphocytes is the "ratio of the number of lymphocytes daily entering the blood through the thoracic duct to number of lymphocytes normally present in the blood [11 6] The D R F however does not represent the total turnover because it does not include cells which enter the blood stream directly from lymphoid tissues[893] or those which may remain in the connective tissue several days, as found by Clark and Clark (1930) [11 6] Yoffey[11 6] gives the value of the D R F for rabbits as 5 0 and for cats as varying from 0 5 to 315 Brilla[110] found in 14 carnivores (wild dogs and foxes) that the average number of lymphocytes was 40 to 55 per cent of the white cells whereas in 17 herbivores (hamsters mice and rabbits) it was 65 20 per cent However this difference may not be as significant as it seems for mice and hamsters are not innate herbivores[534]

Another advantage of the small lymphocyte and other trephocytes is that they contain minerals Cells which release trephones for the use of other cells are therefore a source of tho e minerals which occur primarily intracellularly A deficiency of an essential mineral fraction can be a deterrent to protein synthesis to the same extent as is the deficiency of an enzyme coenzyme essential amino acid or other essential component Nitrogen retention following the acute administration of androgens has been found to have a transient effect in lowering the extracellular levels of potassium and phosphorus [9] There are several fluctuations in nitrogen retention which have parallel effects on potassium and lymphocytes Potassium is stored intracellularly-[79] and only a small amount of this element occurs in plasma[60] Since the number of lymphocytes increases in conditions of nitrogen retention the lymphocyte may be important as a source

phosphatase in small lymphocytes might account for the absence of mitosis in these cells

Numerous other functions have been attributed to alkaline phosphatase, such as its action in transphosphorylation,[1 8] synthesis of DNA,[158] hydrolysis of monesters of phosphoric acid,[991] of glucose 6 phosphate and of glycerol phosphate [69] In addition it is thought that nuclear alkaline phosphatase controls phosphorus turnover in DNA [747]

The presence of alkaline phosphatase in reticular cells of lymph nodes [47] may have a function in P^{3} turnover because Andreasen and Ottesen (1944) *reported that lymphoid organs have a high* P^{3} *turnover* [11 6] *Hevesy and* Ottesen compared the daily renewal of DNA in epithelium by the use of P^{3} and found that 15 per cent of the DNA in the intestinal mucosa was renewed daily compared with 5 8 per cent in the spleen [5] The latter had the second highest turnover activity of the organs studied If such a cycle does occur changes in the Feulgen positive material would not be *noticeable because the DNA could be partly depolymerized and thus* rendered Feulgen negative [899 910] and a comparable amount of the DNA would be resynthesized Al o these possibilities are in accord with the findings that the turnover of DNA in lymphocytes, as shown by the use of radioactive phosphorus, is higher than expected

Alkaline phosphatase in the intestinal mucosa h is been considered by numerous investigators Emmel [34] *noted that this enzyme is regularly* associated with the Golgi apparatus of the intestinal epithelium Doyle [46] also found that the highest concentration of alkaline phosphatase was in the intestinal epithelium bordering the lumen of the appendix in the rabbit

The concentration of alkaline phosphatase along the luminal border of intestinal columnar cells could be due in part to adsorption of the *enzyme from chyle onto the cytoplasmic filaments which form the striated* border to a secretion of the duodenum and jejunum [57] or to being localized on this tissue as a result of being formed within the cytoplasm of the columnar cells Dempsey and Deane (1946) found that the striated border and Golgi zone in the columnar epithelium of a monkey's duodenum were rich in phosphatases [7] However it is apparently not known whether *the alkaline phosphatase in the striated border lies within* and/or surrounds the protoplasmic filaments This is probably due to the finding that ' there is a definite diffusion of the enzyme itself which may give a *false positive reaction* [12 9] Electron micrographs show that the striated border of columnar intestinal epithelium is composed of protopla mic filaments which arise from the cytoplasm within the cell and are thought *to function in the absorption of substances in solution* and in the form of fat droplets 0 5 m in diameter [7] Schachter, Treece and DeLamater[897] describe areas in many intestinal bacteria which stain positively by the

restriction or increased protein catabolism following the excessive administration of cortisone, depletes the thymus first and later the lymph nodes These conditions cause an obvious decrease in cytoplasmic RNA in hepatic cells, and this change occurs before involution of the alveolar cells of the submaxillary gland [35] Depletion decreases the ability of lymphoid tissues to withdraw proteins from body fluids and to retain them by intracellular storage, because there are fewer large lymphocytes which are active in the synthesis and storage of protein

ALKALINE PHOSPHATASE

The distribution of alkaline phosphatase in lymphoid tissue has been a controversial subject because earlier investigators[158, 5, 7] reported finding lymphocytes and lymphoid tissue very rich in this enzyme Gomori points out that most of the earlier investigators believed that the lymphocytes have a high concentration of alkaline phosphatase, but that ' there is no alkaline phosphatase in lymphocytes ' [47] However, Doyle [47] considered lymphocytes a minor source of alkaline phosphatase and stated that they contained only 1/20th as much of this enzyme as the reticular cells This statement was apparently a result of his finding that lymphatic stroma, washed free of lymphocytes, retained 90 per cent of the original alkaline phosphatase activity of entire lymph nodes, most of which was in the reticular cells instead of the fibers

The distribution of alkaline phosphatase in cells coincides with the distribution of DNA in various kinds of cells, such as oocytes of amphibia [5] liver cells[59] and bone marrow cells [8, 1] Danielli and Catcheside (1945) and Krugelis (1946) point out that alkaline phosphatase is restricted to the Feulgen positive bands of the chromosomes [103] Brachet and Jeener (1956) believe that phosphatase is found wherever nucleic acid occurs [880] Arvy and Gabe (1952) report that phosphatase activity was not related to the content of RNA whereas Krugelis (1946) attributed the localization of alkaline phosphatase in the nucleus to the presence of a phosphodiesterase [880] Chevremont and Firket[158] review the reported functions of alkaline phosphatase and mention that Danielli (1946) considered that this enzyme has a function in nucleic acid metabolism that Krugelis (1946) believed that it is specific for the nucleic acid chain and that Brachet and Jeener (1948) noted its relation to phosphorus turnover Chevremont and Firket conclude *that phosphatase is probably 'one of the enzymes necessary* for the synthesis of chromatin or more specifically, of desoxyribonucleoproteins' Ross and Ely[880] noted that alkaline phosphatase had a greater action on lesser polymerized DNA an effect attributed to the action of the enzyme on terminal phosphate groups which increased with depolymerization The ab ence or presence of minute amounts of alkaline

proliferation of low, incompletely differentiated columnar cells in the basal sides of the crypts of Lieberkuhn[193 660] These newly formed cells move sideways out of the crypts and over the denuded areas as prolifera tion proceeds Thus, they replace the lost epithelium Administration of P^3 gives a reaction in crypts of Lieberkuhn which supports the belief that epithelium of villi arises from cells in the crypts [89] As the new cells move outward from the crypts, they differentiate into columnar and goblet cells[91 193 660 746] Bizzozero (1893)[69] and Maximow and Bloom[660] suggest that cells in the gastric pits as in the crypts of Lieberkuhn undergo mitotic proliferation and move over denuded areas to replace lost epithelium

Thus it appears that the mature differentiated, columnar cell in the gastrointestine does not divide, and that the capacity for mitotic activity is limited to cells in certain regions of the gastric and intestinal glands This localization of mitotic activity may not be due to the presence or ab sence of proteins enzymes or other substances but may merely be an adaptation to the structure of the simple, columnar epithelium For ex ample, Drasch (1879) described regeneration of the ciliated cells in the larynx trachea and bronchi as coming through the growth of small basal cells into larger ones that form cuneiform cells which in turn form ciliated cells[6]

Columnar cells of villi are expending energy enzymes nucleic acid and its derivates during the process of absorption of proteins fats and carbo hydrates Macklin and Macklin[6 8] suggest that the absence of mitoses from goblet cells does not point to the inability on the part of parent cells to divide but is related to the probability that a cell which is secreting is not apt to be expending energy in mitosis and that a cell undergoing mitoses is not likely to be forming mucigen The constantly low RNA content of goblet cells is a factor in the inability of these cells to divide for initiation of mitosis is dependent upon increased cytoplasmic RNA Absence of mitosis in goblet cells of the intestine is more likely because the goblet cells are modified columnar cells which normally do not divide

DISTRIBUTION WITHIN THE COLUMNAR CELL

The lymphocytes which occur in the cytoplasm of columnar epithelium of the intestine are usually small lymphocytes We have never seen mitosis in these small lymphocytes However Andrew and Sosa[11] and Andrew and Andrew[10] reported mitosis of lymphocytes in columnar intestinal cells within the crypts of Lieberkuhn in mice We have not been able to verify this observation on small lymphocytes Sometimes a condensation of chromatin in a columnar cell of villi resembles a mitotic figure but these apparent mitoses are often pycnotic or condensed nuclei of spent goblet cells Several investigators however report that lymphocytes in columnar

Gomori technique The variation in the localization and amount of activity was so great, even within different fields of the same culture that the investigators concluded that no cytological interpretation was possible

Several varied conditions alter the amount of alkaline phosphatase in the intestine Tuba and Robinson[1031] subdivided intestinal alkaline phosphatase of the rat into two portions (1) an "adaptive" one which varied with the diet and (2) a "nonadaptive' one which remained constant during fasting Adrenalectomy lowered the alkaline phosphatase in the mucosa of the intestine, but Kutscher and Wust (1943) found that cortisone restored it[104] However, Bourne[96] cautions that there should be awareness that diffusion may and probably does occur" in interpreting changes in alkaline phosphatase Fasting lowered the level of blood serum alkaline phosphatase, but ingestion of fat was followed by a return to normalcy in both rats and men[99]

Lymphocytes in Intestinal Epithelium

The presence of trephocytes within the cytoplasm of the recipient cells is another aspect of nutritional relationship The presence of small lymphocytes in the cytoplasm of intestinal columnar epithelium is a common example of intracellular trephocytes The presence of lymphocytes within the columnar cells indicates that they are related to functions of these cells, rather than being enroute into the lumen of the intestine Lymphocytes in 96 4 per cent of 3117 intestinal columnar cells that were counted in 5 normal hamsters and in 96 per cent of 3478 of these cells in 5 tumor bearing hamsters occupied a position between the nucleus and the basement membrane[516] These values indicate a metabolic relationship between the columnar cells and their intracellularly located lymphocytes, rather than a stage in migration of the lymphocytes into the intestinal lumen

In the relationship between columnar intestinal cells and their intracytoplasmic lymphocytes we have considered the following approaches (1) Small lymphocytes and the columnar epithelial cells of intestinal villi do not normally divide (2) Absence of mitosis in these columnar cells is a modification which permits nucleic and amino acids and their components derived from alimentary proteins to pass from the lumen of the intestine through the epithelium without being used to stimulate mitosis and consequent hypertrophy of the columnar epithelial cells

The absence of mitosis in columnar cells of villi has been acknowledged for many years Hock (1899) Heidenhain (1899) and Greschik (1912) reported that these cells seldom if ever undergo mitotic division however Clara (1926) reports occasionally finding mitosis in the villi of very young chicks[6 8] Repair and replacement of epithelium of the villi is by mitotic

core of villi It is also possible that these lymphocytes may be wandering aimlessly and not have any specific function

The migration of small lymphocytes into and out of the cells could be a regulatory mechanism for the distribution of RNA in the cytoplasm of the columnar cells As indicated in slides stained by the Feulgen reaction these small lymphocytes apparently do not release globules of DNA into the cytoplasm of the columnar cells Dialysis probably plays an important part but the cytoplasmic medium in the columnar cells is very unfavorable for determining whether or not lymphocytic trephones are released by protoplasmic budding However, it was not possible to determine whether a small lymphocyte had recently migrated from the cytoplasm of a columnar cell into the core of a villus or if partly depolymerized DNA, which is Feulgen negative,[639, 910] was released by the lymphocyte into the cytoplasm of the columnar cell in hamsters[534]

Another possible function in the metabolism of the columnar cells is that lymphocytes store and later make available some of the dietary nucleic acid components such as nucleosides,[931] by becoming plasmacytes which are abundant in the core of villi Plasmacytes which are believed to be transformed lymphocytes[493, 633, 660, 759] are very rarely found within the columnar epithelial cells or adjacent to the basement membrane

Small lymphocytes also synthesize RNA and may convert absorbed alimentary nucleotides into RNA within their own cytoplasm or within the cytoplasm of the columnar cell Brown and co workers (1949) found that nucleic acids and nucleotides are utilized in the synthesis of nucleic acids[418] Nucleotides from the hydrolysis of nuclei of small lymphocytes could also be important in DNA turnover in the columnar cells This suggestion is supported by the fact that the addition of DNA which contained isotopic adenine and guanine produced labeled RNA as well as labeled DNA in cultures of chick embryos[619]

Another possible metabolic aspect of the relation between small lymphocytes and the columnar epithelium is an interchange of enzymes Mitochondria which contain many enzymes,[24] occur in 'young lymphocytes' Wintrobe[1103] evaluates the mitochondrial number as high in 'young lymphocytes 10 to 20 in mature and absent in 'old' lymphocytes He states that Potter and Ward (1942) support this tenet by showing that the highest mean number of mitochondria per cell occurs in populations of lymphocytes that have the highest relative number of immature cells Claude[167] observed an increase in the number of mitochondria in lymphocytes following injury

Wiseman (1931) attempted to clarify this classification by stating that 'young' lymphocytes are those that show deep basophilic cytoplasm fol

cells undergo mitosis Bohm, von Davidoff and Huber[91] think that lympho cytes may wander into the epithelium to divide The conclusion of Andrew and Sosa[11] that lymphocytes in the epithelium of the crypts of Lieberkuhn divide is based on the smaller size of some of the mitotic figures in the crypts It does not rule out the possibility that these small mitotic figures could be tangential or peripheral sections of mitotic figures of columnar cells which are most abundant in the crypts of Lieberkuhn

The small lymphocytes in the columnar cells as is characteristic of them elsewhere, have very little cytoplasm but a relatively large nucleus We could not detect any difference in structure or size of the nuclei in small lymphocytes in columnar cells, compared with small lymphocytes in other tissues and blood vessels[516, 5 0, 35]

Small lymphocytes have been observed in the columnar intestinal epithelium of man[660] and most of the laboratory mammals[499] including hamsters[516, 759] (figs 29 to 31) pigeons, [0] several species of salamanders,[737] frogs[893] and many other animals Contrarily Sobotta[9 9] has seen numerous instances of lymphocytes emigrating through the epithelium overlying nodules in the intestine of the rabbit and guinea pig

Fixation often shrinks the core of villi away from the columnar epithelium and forms subepithelial spaces which are considered to be normal by some investigators[6 8] Others however, consider them artifacts caused by agonal changes[6 8]

The use of quantitative methods to determine the percentage of intestinal epithelial cells that contain lymphocytes was not feasible because of great differences in a single individual as well as between members of the control group For example a series of cells containing lymphocytes was often followed by a number of cells which did not contain lymphocytes The number containing lymphocytes fluctuated considerably in a single villus and also between epithelium of adjacent villi[516] Most lymphocytes (about 96 per cent) in intestinal epithelium are in the cytoplasm between the nucleus and the basement membrane and very few occur at the level of epithelial nuclei or between the nucleus and luminal border This distribution of lymphocytes is not due to the volume of cytoplasm because more cytoplasm occurs between the nucleus and the lumen than between the nucleus and the basement membrane—yet approximately 96 4 per cent of the lymphocytes were located in the basal third of the cell[516]

These intracellular small lymphocytes may contribute trephones directly to the cytoplasm of the columnar cells by dialysis or cytoplasmic budding for they apparently do not cytolize Small lymphocytes may remain for a period during which they obtain essential substances necessary for RNA synthesis from absorbed material then migrate into the lamina propria and transform into plasmacytes which are most conspicuous in the

fat into the lymph Absorption of other substances is also credited to the versatile 'lymph corpuscles'

Some writers[41, 898] suggest that leukocytes (presumably including lymphocytes) pass into the lumen of the intestine incept fat particles and return to the lymphatics with the load of fat This tenet is favored by the statement that "white blood cells actually migrate back and forth between the lymph and the lumen of the intestine,"[41] presumably to transport fat droplets and iron to the lymphatic system

Although it is not always clear which 'leukocytes' are involved, in general, Schafer's[898] ideas are supported by Zawarykin (1883) Clark and Clark (1911) Mottram (1923) Mottram, Cramer and Drew (1922) and others, the opposing view is taken by Grunhagen (1887) Heidenhain (1888) and Raymond (1904) who deny any digestive function so far ascribed to lymphocytes[51, 6, 8]

LYMPHOEPITHELIA

Considerable numbers of lymphocytes actually pass through the lymphoepithelium into the flask shaped mucous glands of the rabbit's appendix[70] and through the lymphoepithelium that covers Peyer's patches and other intestinal lymphoid tissues Lymphoepithelium is formed by modified columnar cells of the involved abortive villi being infiltrated by lymphocytes from the underlying nodule Lymphocytic infiltration of this epithelium begins in newborn rabbits and has distorted the regular form and arrangement of the columnar cells by the 3rd day after birth A few solitary lymphocytes are found in the epithelium of the flask shaped glands and villi in rabbit embryos 19 days postcoitus[97, 51] but the total extent of lymphoepithelium represents a very small percentage of the surface area of the intestine

Farr[594] used fluorescent dyes in an attempt to determine if any lymphocytes pass through the intestinal epithelium into the lumen but he was unable to draw a conclusion from the experiments because debris in the lumen of the intestine was also fluorescent However he states that even if these cells left the organism by the intestine this means of removel from the blood would account for only a few of the great number of lymphocytes that disappear in the bone marrow and lymphoid tissues Farr's inability to demonstrate the passage of lymphocytes into the intestinal lumen suggests the possibility that the lymphocytes may undergo cytolysis within the columnar cells or transformation within Peyer's patches or the stroma of villi The absence of thymonucleic acid in the cytoplasm of columnar cells that contain small lymphocytes indicates that nuclei of lymphocytes were not disintegrating in the manner reported for the ovum of *Triturus viridescens*[604]

lowing Wright's stain and many mitochondria after supravital staining, and that there is also a decrease in RNA content of the cytoplasm con comitant with the decrease in number of mitochondria during aging [1103] The methods used in our study of small lymphocytes in intestinal columnar epithelium of hamsters did not indicate a significant difference in baso philia in the cytoplasm of either the small lymphocytes or of the columnar cells in which lymphocytes occurred [516]

It is extremely difficult to visualize functions of the lymphocytes which occur in the lumen of the intestine, since a number of questions are involved on which there is surprisingly little direct information The little known functions and fate of small lymphocytes gave rise to a number of theoretical explanations of the presence of these cells within columnar epithelial cells and lymphoepithelium of the intestine Older histologists[91, 898] described lymphocytes within or between intestinal columnar cells and suggested various activities for these migrants Stohr believed that lymphocytes that found in the columnar epithelium had been arrested *en route* to the lumen of the intestine [91] Bohm and colleagues[91] believed that the number found in the lumen is not proportional to the number present in the epithelium and that as yet no leukocytes have ever been observed in the cuticula itself' although some of these cells probably pass into the lumen of the intestine

For many years the presence of lymphocytes in the epithelium of the gastrointestinal tract has been known in man in common laboratory mam mals and in a number of the lower vertebrates The functions of these cells, however, have not been established experimentally and the numerous theories are as diversified as those regarding the function of lymphocytes in the lymphoid tissues throughout the body Early consideration of the function of small lymphocytes in columnar cells covered only those phases of digestion and absorption known at that time However in a recent article on lymphocytes in the duodenal epithelium of the mouse Andrew and Andrew[10] review a number of theories which attempt to explain the presence of these cells (1) The lymphocytes carry a thymic hormone (2) They are the source of enzymes for lipids or nucleins (3) They are *being eliminated from the body* (4) *They are a factor in resistance to* bacterial toxins, to microorganisms or to cancer In addition Berg has postulated that in the appendix of herbivores emigrated lymphocytes play a part in cellulose digestion [99] Others[898] have thought that the num ber of lymphocytes *increased during digestion*

Schafer[898] and others maintained that a phase in fat absorption is chiefly effected by ameboid lymph corpuscles of the villi injesting the fat after it has entered the columnar cells and then migrating into the central lacteal of the villus where these lymphocytes disintegrate and release their load of

cytes are abundant in the submaxillary lymph nodes of rats[40] and are numerous in the retrothymic and submandibular lymph nodes of hamsters[535] The retrothymic lymph nodes which receive lymphatics from the thymus, contain more plasmacytes and have a relatively larger area of medullary cords than most other lymph nodes of hamsters Jordan[494] following the 1943 rules formulated by the American Society of Clinical Pathologists (Committee 1948) freely uses the terms plasmablast and proplasmacyte to indicate 'intermediate stages in the transformation of the ancestral cells into definitive plasmacytes Michels[69] recognizes 'three types of plasma cells containing cytoplasmic inclusions the "eosinophil plasma cell' "plasma mast cell and Russell body cells Campbell and Good[137] state that "the plasma cell is a cytomorphic variant of a number of cell types of the reticuloendothelial system Typical plasmacytes, as described by Marshalko,[137 691] are easily recognized but the identification of non typical forms is often uncertain This is particularly true with regard to cytologically differentiating the closely similar hemocytoblasts lymphoblasts and plasmablasts[494] Pathological terminology sometimes involves additional terms to indicate transitional atypical precursory or various intermediate stages

The actual functions of plasmacytes are little understood because the available information is almost entirely the result of deductions rather than direct observations The high content of RNA in the cytoplasm of plasmacytes and the importance of RNA in protein synthesis substantiate correlations between an increase of plasmacytes in hyperglobinemia and an absence or rarity of plasmacytes in agammaglobulinemia A paralleling correlation exists between an increase of plasmamytes and antibody formation Another function of plasmacytes may be the production of antibodies because most antibodies are gamma globulin Antibody formation appears to be one aspect of the more basic function of this cell in the synthesis and storage of gamma globulins Apparently gamma globulins can function as antibodies but they have a more basic function in synthesizing and storing nucleoproteins The significance of protein storage in plasmacytes depends upon the site of formation because this cell is not normally present in circulating blood or lymph The most obvious function of plasmacytes is nucleoprotein storage This process however may also be important in regulating protein synthesis and mitosis in cells capable of cell division and thereby the plasmacyte may be a cell which partly controls proliferation of other cells Large lymphocytes in lymphoid tissue also retain protein but this process is in relation to storage between the body fluids whereas plasmacytogenesis occurs in a more specifically localized retention

The difference between antibodies and normal globulins is slight Normal

Measurements of carefully controlled camera lucida tracings of 100 of each type of cell enlarged 1800 times showed that the nuclei of small lymphocytes in the cytoplasm of columnar intestinal cells did not differ significantly in size from those in sectioned Peyer's patches, and that lymphocytes in both sites were approximately the same size as erythrocytes in the hamster. Only nuclei of lymphocytes were compared, because it was not possible to delimit the cytoplasm of lymphocytes from that of the epithelial cell surrounding it. Sometimes lymphocytes in columnar cells were separated from the surrounding cytoplasm by vacuole, but these were too infrequent to serve as a basis for the comparisons.[535]

The first ileac Peyer's patch from the ileocecal valve in hamsters was fixed in sublimate alcohol and stained for DNA by the Feulgen method. Other sections from the same block of tissue were stained with toluidine blue and by various other methods to indicate basophilia and to confirm identification.[516] Nuclei which varied in form were not included; only complete circular sections of entire nuclei were measured. Admittedly, this method is not very accurate due to sectioning technique and variation in measuring the maximum diameter, but it served as a standard of evaluation in comparing nuclei of lymphocytes in sectioned epithelial and lymphoid tissues.[516] Camera lucida tracings of 2000 nuclei of circulating lymphocytes showed that these cells have a mean diameter of 5.5 μ with extremes ranging from 3.3 to 8.9 μ in our hamsters.[516] However, other investigators[984] found that the diameter of circulating erythrocytes varied from 2.0 to 9.0 μ in their strain of Syrian hamsters.

PLASMACYTES

Plasmacytes (plasma cells, "plasmocytes," "Russell body cells") characteristically occur in loose connective tissue, especially following partial stasis of blood, lymph or tissue fluid in which a locally increased amount of protein is temporarily retained. However, these cells do not occur in appreciable numbers in freely circulating normal body fluids. Since plasmacytes are virtually non-motile and are characteristically mobile only under certain pathological conditions, aggregates of these cells are formed at the site rather than by infiltration. Plasmacytes were believed by Enderlen and Justi (1910) and Porcile (1904) to be carriers of nutritive material, and Dantschakoff (1905) suggested that plasmacytes in the submaxillary salivary glands absorbed substances from blood and lymph and transmitted them to the alveolar cells.[691]

Plasmacytes usually occur in the medullary cords of lymph nodes.[494, 497, 689] Their abundance in normal animals was emphasized by Jordan and Morton.[497] Jadassohn (1891 to 1893) demonstrated the presence of these cells in the follicles of lymph nodes in man and lower animals.[691] Plasma

decreased or absent in conditions of agammaglobinemia[1 0 35 113] Injection of ribonucleotides caused hyperglobulinemia in rabbits[843] Injection of typhoid antigens into the foot pad of rabbits produced a peak of RNA in the popliteal lymph node which coincided with the maximal formation of antibodies[7] Most antibodies are believed to be gamma globulin, and it has been shown that gamma globulin fractions contain antibodies[410 756] Furthermore, horse serum globulins cannot be differentiated from antibodies in horse serum[411] Hyperglobulinemia is usually accompanied by plasmacytosis[6 3] Plasma cell myelomas usually cause a high blood globulin level with the formation of abnormal proteins[4] Contrarily protein deficiency,[140] prolonged administration of cortisone[740 339] irradiation[1008] and nitrogen mustards[97] reduce the rate and amount of antibody formation, and also decrease the number of plasmacytes[534]

Plasmacytogenesis

The one thing about the origin of plasmacytes on which most investigators are in reasonable accord is that somewhere along the line the progenitors of these cells were derived from mesenchyme Fluctuating conditions may be chiefly responsible for determining the precursor of the plasmacyte Various mesodermal cells have been considered precursors of the plasmacyte particularly those cells which do not have a well established place in an ancestral tree of some other cell or which might be related to wandering cells such as large mononuclears and various lymphoid cells Primarily, the formation of plasmacytes is a process of withdrawing protein from extracellular fluid and storing it intracellularly to aid in the regulation of protein synthesis in other cells

Conditions conducive to plasmacytogenesis result from partial stasis of blood, lymph[494 497] or tissue fluid Plasmacytes develop from lymphocytes in tissues in which there is subchronic or chronic inflammation These cells are also normally abundant in many specialized structures such as splenic and lymph nodal sinuses and in the tunica propria of the intestine They are also present singly or in small numbers in the normal connective tissue of most organs[5 3 691]

Normally temporary lymph stasis may furnish an important stimulus for transformation of plasmacytes from lymphocytes[776 494 497] in lymph nodes and spleen in contrast to the sparsity of these cells in the thymus which has few or no sinuses An increase in protein however is a more nearly direct stimulus for plasmacytogenesis than formation of noxious metabolites[494 497] or some form of toxin as some investigators[4 4 497] appear to believe

Plasmacytogenesis in the spleen uses amino acids and other substances in blood for the synthesis and storage of RNA and gamma globulin

serum globulin has been described as differing from antibodies in the method by which the two ends of the globulin polypeptide molecule are coiled [407] Formerly arrangement of amino acids[140] was believed to be the cause of differences in antibodies and globulin Cryoglobulins which are abnormal plasma globulins, are thought by *Putnam and Miyake*[817] *to differ* from normal gamma globulin and from each other only in the amino acid which is on the *N*-terminal position This conclusion was the result of dividing 20 myeloma globulins into five types which were based upon the *N* terminal amino acid [816]

Plasmacytes are also abundant in those tissues which are believed to be active in the synthesis of proteins other than antibodies RNA is more abundant in cells which are more active in protein synthesis than it is in other cells Only one half to two thirds of the increase of globulins in rabbits that were injected with pneumococci was found by Liew, Chow and Lee to be antibody [101] The cell which produced antibodies was considered to be possibly the same cell which produced serum globulin [1 4] Even if it should be shown that all of the gamma globulins are antibodies the more basic function of the gamma globulins in plasmacytes could still be the synthesis and storage of the amino acids which are present in gamma globulin Terroine[1010] states that the RNA of plasmacytes may govern a proteinogenesis of which the liberated products would contribute to the formation of blood plasma '

The importance of RNA in the synthesis of proteins has been considered by many investigators who used various techniques Caspersson (1941), and Brachet (1942) concluded that RNA has a function in protein synthesis [107] Brachet reviewed the later literature on the cytochemical and *quantitative evidences which indicates that protein synthesis is related to* RNA Microspectrographic studies indicate that cells with high concentrations of RNA in the cytoplasm and nucleus are active in protein synthesis [148] In addition an increase in cytoplasm occurs during protein synthesis [143] However, Brachet (1950) believes that protein synthesis is more closely related to cytoplasmic RNA [30] Nevertheless the means by which RNA functions in protein synthesis has not been determined RNA has also been considered a possible source of energy rich bonds by Spiegelman (1946) [107] Brinkley suggested that RNA has a part in peptide synthesis *Theories on the relations of nucleic acids to protein* synthesis are reviewed by Borsook[94] who points out that RNA plays a more direct part in this process than DNA He also considered the possibility that the carboxyl group may be linked to a phosphate and that the amino group remains free until the formation of the peptide bond in protein synthesis by the template hypothesis

Plasmacytes are increased in states of hyperglobulinemia [5 68] and

Naegeli [691, 559] Drinker and Yoffey [51] state that one obvious fact in considering potentialities of lymphocytes is that they can become plasmacytes Ehrich [75] believes that ' the plasmacyte is a cellula sui generis, and that it arises, not from lymphocytes but from the undifferentiated mesenchyma '

The formation of plasmacytes from lymphocytes has been demonstrated experimentally Partial ligation (producing stenosis) of the aorta between the two renal arteries ("renal ischemia') caused (1) a notable accumulation of plasmacytes at the apex of lymphatic organs, (2) a great increase in the number of plasmacytes and (3) a strong increase in periodic acid Schiff (PAS) positive material in the tip of the thymus Treating these rats with cortisone produced regression of the accumulated plasmacytes and of the increased PAS positive material [1 0] Injections of omatotropic hormone (STH) of the hypophysis produced increased plasmacytes, mast cells, glycoprotein and mucoprotein in lymphoid organs of hypophysectomized and normal rats [1 0] Administration of glucagon ' which is claimed to be truly secreted by alpha cells of the pancreatic islands produced similar results in normal rats [1 0] Thus the results obtained by Cavallero[150] show that lymphocytes may transform into plasmacytes

The mass of evidence and its interpretation supplied by original works and the various, extensive reviews of the literature[494, 497, 6 7] impress one with the potentially multiple origin of plasmacytes They also cause one to appreciate the suggestion that the plasmacyte may represent a functional stage of any multipotent cell and that appropriate stimulation may cause all multipotent cells of the connective tissues to transform into typical plasmacytes [137]

It has also been suggested that the geneaology of the plasmacyte may be related to the type of stimulating factor involved and to the structure of the site stimulated Thus, Campbell and Good[137] found that plasmacytes were formed from invasive lymphocytes from microglia from oligodendroglia and from reticulum cells from the pia arachnoid in experimentally produced herpetic virus encephalitis in rabbits Plasmacytes were formed from ' both von Kupfer cells and the infiltrating lymphocytes in the liver [137] Good and Good (1949) show that splenic plasmacytes arose from reticulum cells macrophages and lymphocytes [137] Although there is virtually nothing known about the quantitative potentialities of the various plasmacyte producing cells it appears that the lymphocyte is the one cell most often identified as being directly in line as a progenitor of the true, or Marschalko s plasmacyte However sharp distinction between lymphocytes and plasmacytes cannot be made because there are intermediate cell stages which cannot be classified as typical lymphocytes or typical plasmacytes Immature or young plasma cells are considered by Weiss[38] and by Keunig and Van der Slikke [39] to be producers of antibodies Furthermore, the

specifically for first use by hepatic cells In chronic inflammation and wound healing plasmacytes store nucleoprotein during the period between the stages of initial hyperemia and regeneration Plasmacytes in the salivary glands, and under certain conditions in the mammary glands store protein to bridge the time interval between periods of inactivity and activity of these glands Plasmacytes in the bone marrow of mammals store nucleoproteins for use by hematopoietic cells Those in villi of the intestine situated between the intestinal lumen and the hepatic portal vein, store alimentary proteins to be passed directly to the liver when needed, those in the medullary cords of lymph nodes, which are functionally situated between the lymph and blood, may pass their stored proteins over efferent lymphatics to the thoracic or cervical duct and thence into the systemic circulation

There is strong evidence that plasmacytes develop from lymphocytes but their origin remains controversial Nevertheless, the possibility exists that some cells which may be identified as plasmacytes are derived from other stem cells This is because the identifying characteristics of plasmacytes are nondifferentiated properties such as a high content of homogeneously dispersed cytoplasmic RNA which characteristically occurs in earlier stages of several erythropoietic and myelopoietic cells Michels and Globus[69] reviewed the literature beginning with Unna s (1891) work They report that various investigators have believed that plasmacytes arise from tissue lymphocytes (emigrated small lymphocytes) but that others maintain that large lymphocytes various monocytes fibroblasts wandering cells hemohistioblasts adventitial cells monocytes hemoblasts, myeloblasts granuloblasts (all of which may be classed as monocytes),[746] erythroblasts[691] and probably other cells or cell stages may also become plasmacytes Several types of mesenchymal cells have been considered precursors of plasmacytes particularly those cells which do not have a well established place in an ancestral tree of some other cell or that might be related to any of the reticuloendothelial mesothelial endothelial, hematological or fibroblastic types Primitive connective tissue cells [663] possibly large mononuclear cells [90] and adventitial cells of small blood vessels [7] have also been considered sources of plasmacytes

Most of the more recent writers believe that plasmacytes usually arise from mature or small wandering lymphocytes by transformation [43 51 3 4 497 633 65 636 691 75 1103 11 6] Plasmacytogenesis by transformation of lymphocytes is disputed by a number of investigators Jordan and Morton (1937) believe that it is doubtful whether or not plasmacytes can be derived by transformation of lymphocytes [11 7] However it is generally conceded that plasmacytes are derived from lymphocytes as was proposed in Marschalko s theory of 1895 which is strongly supported by Piney Downey and

Cajal a year before Unna described these cells,[69] and was used by him in identifying plasmacytes instead of the spoke like arrangement of nuclear chromatin which Marshalko proposed as the identifying characteristic.[691] Sylberza (1941) also thought that ribonucleoproteins caused the basophilia of plasmacytes[75] but Ferrata did not consider cytoplasmic basophilia as a distinguishing characteristic of these cells.[691]

ATYPICAL PLASMACYTES

Typical plasmacytes are readily identified but atypical cells which are numerous are difficult to identify, especially the earlier stages of development. Some investigators recognize two or three early stages, the more common of which are called the proplasmacyte and the plasmablast.[75, 493] Ferrata and his colleagues apparently ruled out Türk's irritation cells on the grounds that nuclei of these cell forms differ from those of plasma cytes and he interpreted Türk's cells as 'developmentally inhibited hemocytoblasts'[691] instead of disintegrating plasmacytes. Various terms have been introduced to designate aberrant atypical immature various 'physiological,' transient and other forms of plasmacytes. Michels[691] ably reviews the literature and gives the source and application of such terms as erythroblastic, lymphoblastic, monoblastic and myeloblastic plasma cells and undifferentiated erythroblasts or granuloblasts, clasmatocytes, adventitial and resting wandering cells, large mononuclears and various lymphoid cells, as precursors of plasmacytes. Michels[69] recognized three types of plasma cells containing cytoplasmic inclusions: the eosinophil plasma cell, plasma mast cell (Neutrophil) and the Russell body cell. Jordan[494] following the 1943 rules formulated by a committee of the American Society of Clinical Pathologists in 1949 (Committee, 1948), used the terms plasmablast and proplasmacyte to indicate intermediate stages in the transformation of the ancestral cells into definitive plasmacytes. Campbell and Good[137] state that 'the plasma cell is a cytomorphic variant of a number of cell types of the reticuloendothelial system. Typical plasmacytes as described by Marshalko[137, 691] are easily recognized but as most authors intimate identification of non typical forms is often uncertain. This is particularly true with regard to cytologically differentiating the closely similar hemocytoblasts, lymphoblasts and plasmablasts.[494]

Downey (1911) and certain other investigators believe that some of the morphologically atypical plasmacytes are derived from large mononuclears, Maximow's resting wandering cells (clasmatocytes), large lymphocytes of the leukocytoid type and possibly from a few fibroblasts.[691] Almkvist (1910) Schlesinger (1902) and Pappenheim (1919) advocated the recognition of two types of plasmacytes: Unna's plasma cells and

lymphocyte has been considered as a possible cell to which globulins are transferred in order to receive the antibody stamp [1 4]

CYTOPLASMIC BASOPHILIA

Plasmacytes have the cytoplasm well supplied with free trephones and with trephone yielding organelles The cytoplasm of plasmacytes contains mitochondria, usually clustered around a centrosphere that includes Golgi apparatus of Cajal, which are surrounded by a few neutral red positive vacuoles [603] Michels[691] reviews ideas on the presence of granular cytoplasm and states that Kingsley (1924) mentioned a ' homogenous granuloplasm as the first sign in the formation of plasma cells from lymphocytes Jadas son suggests that the cytoplasm of plasmacytes is crumbled rather than granular, whereas De Asua (1922) believes that the crumbled form is the result of an accumulation of secretion [691]

Descriptions based upon the use of the electron microscope confirm the earlier descriptions of the granule like structure of the cytoplasm of plasmacytes Ehrich[275] used the electron microscope to show that a bundle of filaments occurs in the cytoplasm of plasmacytes and was considered a framework of ribonucleoprotein that is the factor which produces protein for export ' Granules which were usually spherical 80 to 300 Å in diameter and had a relatively high electron density were abundant in the cytoplasm of plasma cells [73] Long parallel or concentric or sometimes branching lamellae have been considered the reason for cytoplasmic basophilia of plasmacytes [1] Braunsteiner and co workers (1953) and Policard and associates (1954) have shown that archoplasmic lamellae form most of the cytoplasm of plasmacytes

RNA accounts for the greater amount of basophilia in the cytoplasm of plasmacytes [10] Harris and Harris found that 2 days after injecting antigen into rabbits the plasmacytes contained more RNA than most cells in sections or imprints when treated with the RNAase histochemical test or by the use of methylene blue solution at pH 4 9 a method which demon strates RNA [485] Plasmacytes contain more RNA than pancreatic alveolar or hepatic cells or Perkinje neurons [534] Brachet[10] is convinced that the *basophilic substance in the cytoplasm of lymphocytes is ribonucleoprotein* Jones[491] points out that there are various types of basophilic cytoplasm and that the work of Caspersson and Schultz using ultraviolet absorption spectroscopy and of Brachet using RNAase methods shows that cyto plasmic basophilia is due to the presence of ribonucleoprotein By the use of RNAase Brachet successfully deprived primitive and definitive erythro blasts lymphocytes and myeloblasts of cytoplasmic basophilia which he identified as being caused by ribonucleoprotein [491] Cytoplasmic basophilia is a property of most *plasmacytes* [3 437 493] *and was noted by Raymon y*

the posterior half of the duodenum than in the jejunum or ileum, except in the last inch of the ileum where they are abundant[534] due to intermittent closure of the ileocecal valve[4 3] which increases the volume and protein content of the chyle by stasis

Mild inanition reduces the total number of plasmacytes in the intestinal villi, whereas prolonged hunger may practically deplete the villi of these cells [10] Six hamsters which were supplied with drinking water *ad libitum*, but received no food for 3 to 5 days, showed a pronounced reduction in plasmacytes in the core of duodenal and ileal villi [7 10] In two groups of 5 hamsters each of which received no food until one animal in each group was near death the core of all villi contained very few plasmacytes in all 10 of the animals In 15 hamsters maintained from 10 to 36 days on a protein free but otherwise adequate synthetic diet to which 2 per cent (dry weight) Brewers yeast was added the villi in the ileum had a notable decrease in each animal However, only 4 of the 15 hamsters showed reduction of plasmacytes in duodenal villi [10 534] Subpanniculur injections of human gamma globulin (0 5 to 1 0 ml 6 to 20 injections during 6 to 47 days) greatly increased the number of plasmacytes in the villi of the duodenum and in many villi in the ileum in all of the 5 hamsters [10]

In general any systemic alteration in nitrogen is reflected as a corresponding change in the number of plasmacytes in the villi [34] Thus it was not surprising to find that subpanniculur injections of cortisone acetate (Merck, 10 0 mg/k 3 to 18 times during 5 to 41 days) into hamsters not only reduced the number of plasmacytes in the villi of all 13 animals sharply, but al o cau ed variable dilation to actual ballooning of an appreciable number of villi in some instances This distention occurred in the lacteals and appeared to be a result of decreased or lack of tonus and possibly increased lymph pressure in the villus (fig 25) However it was shown that plasmacytes in the core of villi were more slowly destroyed than those in Peyer s patches in 29 hamsters which received 850 to 995 r total body x irradiation In 13 of the hamsters killed within 10 days subsequent to the radiation the number of plasmacytes in the villi was approximately normal but the Peyer s patches had been practically destroyed

Glandular Activity

There appears to be a definite correlation between the number of plasmacytes and mast cells in the adjacent connective tis ue and the activity of the gland in question This is especially noticeable in those glands subject to alternating high and low levels of activity in which the activity characteri tically reaches a crest of comparatively short duration followed by a longer period of greatly reduced activity or of virtual inactivity

Marschalko's "plasma cells"[691] Use of the electron microscope indicated that Mott cells may be plasmacytes whose cytoplasm contains dilated sacs[75]

Plasmacytes are often considered inflammatory cells[71] Pappenheim (1919) thinks that plasmacytes are "inflammatory, altered histogenous lymphocytes of granulation tissue", Weidenrich (1909) thinks that since the plasmacyte is a transient "irritative physiological condition, it may occur in various lymphoid cells," possibly including lymphoblasts[691] Mc Gowan (1926 1927) carried Weidenrich's idea that plasmacytes represent a morphological rather than a physiological entity further by postulating lymphoblastic monoblastic, myeloblastic and erythroblastic origin of certain plasmacytes[691] apparently to indicate that these were merely immature, or blast cells which had assumed the general morphology of other cells Certain investigators have interpreted plasma cells as aborted hemoblast[691]

PLASMACYTES IN VILLI

Plasmacytes in the core (lamina propria) of intestinal villi apparently obtain the protein they store chiefly *from chyle* rather than blood In this structure, plasmacytes are synthesizing and storing protein in an intra cellular form between the columnar epithelium and the blood capillaries and lacteals of the villus (figs 29 and 30) A condition approaching stasis in villi could hardly exist because of the extensive precapillary arterio venous anastomoses which have been demonstrated in man and the rab bit[47] In addition the practically ceaseless movement of the villi would effectively prevent any degree of stasis and practically preclude exudation *within these structures*[534] *It thus appears that the great number of plasma cytes in the lamina propria of the villi (figs 29 and 30)* results from ab sorbed dietary amino acids rather than from plasma protein, that stimu late the transformation of lymphocytes into the numerous plasmacytes found in intestinal villi There are wide variations in the number of lympho cytes and plasmacytes in the core of intestinal villi in different species and even in different loci in a single hamster[16 534] However the plasmacytes are storing more RNA and gamma globulin per cell than are the small lymphocytes They are functioning in the same relation of body fluids to organs as are those lymphocytes which occur in Peyer's patches solitary follicles and other intestinal lymphoid tissues

Plasmacytes are more abundant in the core of intestinal villi in hamsters than in the villi of mice[14] The number of lymphocytes and plasmacytes in an individual villus of a hamster varies *according to the location in the* intestine of the villus and its proximity to intestinal lymphoid tissue Plasmacytes are more numerous and more evenly distributed in villi in

function as trephocytes that supply necessary components for the proliferation of interstitial connective tissue and for the secretions of the alveolar cells During the *involutionary phase (in man) there are infiltrations* of lymphocytes[100] which Aschoff[6] regarded as indicating 'so called retention mastitis' but which Boyd[100] believes is functioning in the 're*moval of cell debris and secretion' rather than indicating the presence of* inflammation Fifteen years before Boyd s statement Taylor[1014] expressed the belief that 'round cells that infiltrate the breast are a part of the *semiphysiological mechanism of resorption of secretion in the breast and* prostate as a result of necrosis' in hyperplastic or rapidly proliferating tissue

Prelactating Gland Growth in the mammary gland is initiated partly by histamine induced vascular changes which are similar to growth processes initiated by hormones in other organs Loeb[61] states that growth is associated with intensified circulation and increased blood supply, and that growth in the mammary gland is due to the dilation of capillaries and the formation of a capillary field around newly forming tissue The blood supply of the mammary gland is abundant[493 746] and greatly increases as function develops[77 660] Lymphatics are also numerous in the interalveolar and interlobular connective tissue[91] and pass chiefly to the axillary lymph nodes[91 14]

Lymphocytes are present at all active stages of normal growth or hyperplasia of the mammary gland as shown by Schroder and others[1014] Infiltration of lymphocytes and plasmacytes accompanies any rapid proliferation of glandular or surrounding interstitial connective tissue in the mammary gland[100 615 660 811 1014] The infiltration of lymphocytes into the connective tissue of the mammary gland during pregnancy in women[100 615] usually occurs during the proliferative phase of the gland[100] and at the same time that hypertrophy of mammary epithelium occurs[660] Berka also observed round cell infiltration in the hypertrophied mammary glands of newborn children[1014] An infiltration of lymphocytes occurs in the mammary gland of women at the end of the lactation[100]

Estrogens are generally considered the causative factor for increased circulation in the mammary gland[57 393] but their effect as a direct stimulator of growth has not been established It is thought that estrogen increases vascularity in the mammary gland by having a local effect—and *increases the permeability of the vascular bed to other hormones in the* blood[393] However other investigators[218 331 615] believe that the effect of estrogens is mediated through their effect on the pituitary Curtiss[18] observed species differences in the effects of estrogens upon growth *of the* mammary gland He found that complete lobuloalveolar growth followed the administration of estrogens in the cow goat and guinea pig but that

GENERAL CONSIDERATIONS

The number of plasmacytes and the synthesis of protein in certain glands fluctuate in response to intermittent periods of secretory activity and inactivity Plasmacytes are numerous in the connective tissue in the normal pancreas salivary and mammary glands These glands produce copious amounts of protein containing secretions at intervals followed by periods of reduced activity or rest The blood supply in the salivary glands is probably inadequate to meet the heavy, intermittent demands Thus, it becomes necessary for these glands to draw upon the AMP and histamine, RNA and other substances and components stored in mast cells, lymphocytes and plasmacytes in the connective tissues in order to maintain physiological efficiency of the secreting cells and to obtain the essential components for the production of normal saliva

Plasmacytes in the pancreas are rare within the islands of Langerhans but occur singly throughout the interalveolar connective tissue The rarity of plasmacytes in the islands of Langerhans may be due to the fact that islet cells have comparatively little cytoplasmic RNA as compared with alveolar cells In addition, the islands of Langerhans have a well developed and highly vascular capillary network which apparently, is controlled by the release of histamine stored in the granules of mast cells [3, 33]

The thyroid gland differs from the islands of Langerhans and the anterior pituitary because the capillary network is not so closely related to the secretory cells This difference in relations of the capillaries and the probability that the plasmacytes supply a certain amount of globulin for the formation of thyroglobulin may account for the greater number of lymphocytes and plasmacytes present in the connective tissue associated with the thyroid gland than occurs around nonalveolar glands such as the anterior pituitary and islands of Langerhans

MAMMARY GLANDS

Infiltration of lymphocytes and the transformation of some of them into plasmacytes occur in the mammary gland in normal physiological states in which the plasma proteins are increased in the connective tissue During pregnancy and before lactation plasmacytes synthesize and store RNA globulins and other proteins for use by the alveolar cells of the mammary gland during lactation Some of these plasma cells disintegrate to provide trephones for proliferating alveolar cells and others become colostrum corpuscles At the termination of lactation the plasmacytes synthesize and store RNA and proteins from the debris of the involuting alveolar cells for controlled release into the blood and lymphatic systems

Plasmacytes in the mammary gland have two important but different functions during the mammary cycle During the secretory phase they

them as histiocytes, monocytes or mononuclear leukocytes[615] Maximow and Bloom[660] believe that they are more probably, lymphoid cells which ingest fat droplets and get into the lumen of the glands More recently, the colostrum corpuscles have been considered to be modified plasma cytes[811] The extensive distribution of plasmacytes in inflammatory and diseased conditions of the udder of the cow suggested to Porter[811] that plasmacytes may be responsible for the production of the high protein concentration in colostrum and mastitic milk "

If plasmacytes are one, or the only source of colostrum corpuscles, the presence of fat globules in the cytoplasm represents another modification in addition to altered protein and carbohydrate metabolism which occurs in the metabolism of plasmacytes Several types of protein inclusions have been described in the cytoplasm of abnormal plasmacytes Examples are Auer like bodies in multiple myeloma [97] and Russell bodies which are supposed to occur in degenerating plasmacytes[691] Some which give a positive PAS reaction appear to be mucoprotein [798]

The high lactalbumin [9] and gamma globulin content of colostrum in cattle[811] suggests a direct utilization of gamma globulins from plasmacytes Furthermore during the period of colostrum formation, extensive infiltration of small lymphocytes and polymorphonuclear leukocytes and monocytes as well as plasmacytes, occurs throughout the epithelium In this case the direct source of gamma globulin in the colostrum would be the plasmacytes and probably would not involve resynthesis by the alveolar epithelium of the gland This is important because of the sudden increase in gamma globulin during the period of colostrum formation Obviously it would be very difficult for the alveolar cells of the mammary gland to secrete sufficient quantities of gamma globulin if the source were limited to plasma globulin The high increase in gamma globulin and antibodies in colostrum represents (1) a prolonged synthesis of the gamma globulins during the period of pregnancy (2) their storage within plasmacytes and (3) the direct disintegration of the plasmacytes in the lumen of the gland The development of plasmacytes in the stroma of the mammary gland and the synthesis of gamma globulin during pregnancy are processes by which gamma globulins can be synthesized in considerable quantities over a long period of time

Disintegration of plasmacyte within the lumen of alveoli might explain the extremely high content of gamma globulins and in turn of antibodies[811] during the period of colostrum formation and the subsequent decrease in these substances during the period of lactation when there is relatively little infiltration into the glandular stroma by lymphocytes and plasmacytes During lactation amino acids are probably used directly by the alveolar cells to synthesize mild protein whereas during pregnancy

only growth of the ducts occurred in the rat, mouse and rabbit. Progesterone was necessary for lobuloalveolar growth in these laboratory animals. The effect of estrogens in increasing the permeability of blood vessels has also been considered a cause of edema in the uterus of mice.[9]

An increase in vascularity and permeability occurs in the mammary gland during its development and in puberty,[393, 1014] during the menstrual cycle,[660] and during pregnancy and lactation. The glandular stroma becomes somewhat edematous during the menstrual cycle in women.[615, 660] However, hyperplasia of epithelium in the ducts and acini following the hyperemia and edema of the menstrual cycle has not been established.[660] There is an increase in blood vessels in the interstitial connective tissue between small alveoli and underlying secretory portions.[660] Dempsey, Bunting and Wislocki[3] believe that basket cells have a function in altering the circulation by becoming stellate and thereby forming an open membrane into which capillaries move as the spaces appear. This rearrangement places capillaries immediately adjacent to the epithelium of the ducts.

There is considerable evidence that estrogen and progesterone are indirectly responsible for the initiation of the hyperemia and subsequent hyperplasia of alveolar cells in the prelactating mammary gland in hamsters.[5, 9] The direct effect of these two hormones is chiefly to induce mastocytogenesis, substances released by the thus formed mast cells, mainly histamine and heparin, initiate alveolar hyperplasia by causing hyperemia and increasing capillary premeability with protein leakage into the intercellular fluid (see Chap. 5 and 6).

Lymphocytes and plasmacytes aggregate in the mammary gland before secretion of colostrum, at the beginning of lactation and at the termination of lactation. The formation of plasmacytes before and during development of colostrum[615] is responsible for the tenet that colostrum corpuscles form plasmacytes. The coincidence of colostrum formation with the presence of heavy lymphocytic infiltration and usually plasmacytic aggregation in the interstitial connective tissue that surrounds the alveoli and lobules of the gland[615, 660, 811] appears to indicate that much of the protein that is concentrated in colostrum, which is not derived through hyperemia, is obtained from the infiltrating lymphocytes and aggregating plasmacytes.

The origin of colostrum corpuscles has been a subject of controversy. Several investigators state that colostrum corpuscles are apparently derived from lymphocytes.[31] Others[498] believe that some of the colostrum corpuscles actually are lymphocytes. Some[660] imply that plasmacytes may be a source of colostrum corpuscles because their appearance in the mammary gland is coincident with the appearance of colostrum. Gregoire believed that colostrum bodies were epithelial cells.[403] Gruber (1924) classed

same steps as those in chronic inflammation. Pichard states that the steps in the production of chronic cystic mastitis include (1) mechanical stasis, (2) round cell infiltration, and (3) proliferation of epithelium.[60] These three conditions are similar to those present in chronic inflammation in general. The predominance of plasmacytes in this condition occurs in a tissue fluid which is formed as an exudate that has a high protein content.

Mammary dysplasia is a nonbacterial pathological condition which results from the 'failure of reciprocal proliferation and involutions' and is 'essentially an abnormal interplay of parenchyma and stroma.'[573] Kuzma[73] believes that the anatomical changes in the gland are due to prolonged ovarian hormone imbalance, such as deficient corpus luteum with absolute or relative hyperestrinism. An increase in estrogens has been shown to produce hyperemia and edema in the mammary gland.[493, 530, 61, 746]

amino acids in the blood can be synthesized and stored in the form of globulin in the cytoplasm of plasmacytes Amino acids in the blood during lactation are used by the columnar cells of the alveoli to synthesize caseins and lactoglobulins The same amino acids occur in beta lactoglobulin and in alpha and beta casein as occur in gamma globulin, but the amounts vary[410]

Plasmacytes are not as numerous during lactation as at its termination[535] This observation supplies additional support for the idea that the function of plasmacytes is to bridge the protein gap[53] From a cytological standpoint this is extremely important in view of the aggregation of plasmacytes in the mammary gland during various stages of pregnancy and at the termination of lactation In making these comparisons, we do not maintain that the gamma globulin molecule that is derived from plasmacytes is directly incorporated in the milk At certain stages the high gamma globulin content may represent the same simple proteins which are synthesized by plasmacytes

Cessation of Lactation The infiltration of lymphocytes and the aggregation of plasmacytes in the mammary gland at the termination of lactation probably constitute a resorptive mechanism for salvaging decomposed material or secretions[100, 60, 1014] Chronic inflammation that results from milk stasis is often thought to cause this "round cell infiltration Several authors recognize the fact that the infiltration is not due to infection[811] Taylor[1014] considered round cell infiltration more probably related to resorption and secretion than to an inflammatory reaction This idea is supported by our investigations[34] Also lymphocytes alone have been credited with the removal of secretions and disintegrating cells[100]

Infiltrations of lymphocytes and the formation of plasmacytes at the termination of lactation occur in tissues which have a high protein content This protein is derived chiefly from (1) cytolysis of alveolar epithelium (2) the pooling of secretion in the lumina and (3) the increase of extravasated protein in tissue fluids Thus the formation of plasmacytes from lymphocytes in the mammary gland at the termination of lactation should be regarded as representing synthesis and temporary intracellular storage of growth promoting substances which permit slower passage of these substances into the blood

Several nonbacterial pathological conditions of the mammary gland are characterized by heavy infiltrations of lymphocytes and plasma cytes[811] Plasma cell mastitis occurs in conditions of stasis and inspissation of the secretions which are considered to be the causative factor[5 4] Plasmacytes are the predominant cells but giant cells and foam cells are also present grouped about lipoid crystals[573] It is significant that changes in the mammary gland in chronic cystic mastitis follow essentially the

Mast cells are said to be capable of ameboid movement[693] and, consequently, of diapedesis.[133] They occur in subacute inflammations[96] but are not phagocytic.[597] Bloom and co-workers,[88] with the aid of the electron microscope, observed "a large number of small filamentous protoplasmic protrusions" on the surface of mast cells in a mastocytoma of a dog. At least two investigators claim that mast cells along capillaries pass granules and certain other substances directly into the blood stream by means of their cytoplasmic processes which are inserted between the endothelial cells into the lumina of the capillaries.[693] In vascular injury these perivascular mast cells pass heparin through the walls of blood vessels to aid in forming a protective film over injured endothelium and to prevent the formation of a blood clot at the site of the injury.[695] However, certain investigators contend that mast cells do not possess the power of ameboid locomotion (or of any other mode of locomotion for that matter) and that they are incapable of penetrating capillaries, of phagocytosis or of true secretory activity.[693] They further contend that there is no agreement on the morphology, function or origin of these cells.[51, 693] Jernstrom[486] states that "there is still no unanimity of opinion as to their origin or function in man, although mast cells have been studied rather extensively since they were recognized in 1863."

Sehrt (1927) believed that mast cells fix oxygen by virtue of their lipoid and oxidase content, and other investigators also regarded them as carriers of oxygen.[693] The increase in number of mast cells in the mammary gland during initiation of lactation suggested to Higuchi (1930) that mast cells may have some indirect relation to secretion.[693]

ORIGIN AND DISTRIBUTION

Mast cells occur in almost all of the structures in the body.[693] Characteristically, if not altogether, they occur in loose or areolar connective tissues[494, 535, 693, 109] and in many different species.[60, 693] However, they are rare in parenchymatous organs such as the liver, kidneys and suprarenal gland. Mast cells are absent in cartilage and bone but are present in marrow, perichondrium and periosteum[693] which have little loose connective tissue. Mast cells are also common in arthropods and occur in most astonishing numbers in fish tissue.[8]

Various investigators believe that in both the higher and lower vertebrates mast cells may arise from tissue hemohistioblasts (resting wandering cells), tissue lymphocytes or indirectly from globule leukocytes. In addition, mast cells may arise from blood mast cells, especially in lower vertebrates.[693] Baumer (1896) thought that mast cells were developed from precursors (presumably endothelial cells) in the wall of arterioles and

3

Mast Cell Interrelations

Mast cells have been shown to store heparin[500, 856, 1045, 1055] histamine[63, 71, 854, 1055] and possibly serotonin[[illegible], 6, 884]. The fact that products of mast cells occur in spherical granules which are widely dispersed after lysis of the cell (figs. 1 and 7) increases the efficiency of these cells in storing and in distributing trephones and pharmalogical substances as compared with those trephocytes which liberate their products in the form of a colloidal or true solution. *The intact granules of mast cells become scattered throughout a considerable area of tissue*[10, 6] and each granule becomes a center of activity instead of the cell forming the center of dispersion of its products.

Mast cells have been called basophile tissue cells, basophile granulocytes,[336] heparinocytes,[464, 493] histaminocytes,[860] histogenous mast cells, leukocytes with gamma granules (Ehrlich 1879), mast cells of Ehrlich, mucinoblasts of Harris,[693] mastocytes,[1, 0] tissue mast cells and other names. Ehrlich (1877) maintained that the cells described by Waldeyer (1875) as "plasma cells" were really mast cells.[99] Ehrlich introduced the commonly used term "mast cell" in 1879 because he believed that these cells represented over nourished connective tissue cells.[693, 99] However, it is sometimes impossible to determine whether a statement concerns tissue mast cells or circulating mast or basophilic leukocytic cells because of overlapping and otherwise confused use of terminology. Incidentally, this *imposing array of terminology presages the extensive and highly controversial literature on mast cells.*

The morphology of mast cells in normal animals as shown by camera lucida tracings is highly variable[693, 6[illegible]]. The cells are irregular to spheroidal in form and characteristically densely packed with large spheroidal basophilic cytoplasmic granules when they are mature (fig. 1). The granules commonly stain metachromatically with most basic aniline dyes[336] and are usually so numerous that they obscure the nucleus. Mast cells are highly polymorphic *when subjected to high hydrostatic pressure in the peritoneal fluid of young female rats treated with colchicine.* The polymorphism is attributed to the effect of colchicine on the cytoplasmic viscosity.[66]

cells into mast cells The degenerating collagen apparently stimulates the transformation with the result that certain components of the collagen, especially AMP and sulfur are stored within the forming granules of the developing mast cells Thus it appears that the chief function of these mast cells is to salvage material from the degenerating collagen rather than to contribute to the cause of such conditions as subchronic inflammation, myxodema fungoides and lupus erythematosus in which these cells are greatly increased in number[630]

Although speculation is rife little is definitely known about the genesis of mast cells This is chiefly *because investigators have been unable to* establish reliable criteria for the recognition of the pregranular stages which do not absorb fluorescent stilbamidine[8] and do not stain metachromatically as do mature mast cells

Mast cells appear to be derived chiefly by the transformation of undifferentiated mesenchymal cells which are always fairly abundant in the loose connective or areolar, tissue It is in this tissue that mast cells are most abundant in both young and adult animals It is generally believed that in the differentiation of the various special types of connective tissues from mesenchyme many of the mesenchymal cells remain indefinitely undifferentiated[393, 493] Also undifferentiated mesenchymal cell derivatives of the fixed type like mast cells are distributed chiefly in the loose connective tissue along blood vessels[393, 00, 8, 1004] Thus, both the developmentally potential undifferentiated mesenchymal cells[660] and mast cells assume a conspicuously perivascular distribution which is taken, like perivascular infiltration of the liver in tumor bearing hamsters[06, 0, 53] to indicate a very close relationship which is probably transformation

Earlier work showed that the more extensive degenerative changes were accompanied by a larger proportion of mast cells[691] This correlation supports the contention that mast cell granules may liberate histamine which aids in repair processes by causing local hyperemia with increased capillary permeability That mast cells store components of degraded collagen in their granules is suggested by the increase in number of these cells in the mammary glands in which collagen was replaced by fat in normal hamsters[530] and by association with the destruction of collagen fibrils in all advanced cases of disseminated lupus erythematosus[4]

Mast cells are present in large numbers in the peritoneal cavity especially in the serous fluids of *Triton cristatus* (a salamander) rats cats and certain other animals (fig 1)[693] They are rare or absent in the peritoneal fluid of rabbits[693] We regularly find numerous mast cells in the peritoneal fluid mesenteries and retroperitoneum of normal and tumor bearing hamsters

probably, of other small blood vessels[85] The Dalgaards (1948) apparently advocate a similar, although somewhat modified, paraepithelial origin with subsequent migration into the perivascular connective tissue[85] However, it is generally conceded that undifferentiated mesenchymal cells or reticuloendothelial cells are the chief source of mast cells,[393 660 85] but they "may arise heteroplastically from lymphocytes plasma cells, clasmatocytes and histiocytes through endogenous elaboration of mast granules"[3] Bates[51] concludes that 'the predominant interpretation favors a possible origin from the lymphocyte" Nevertheless, he takes issue with this view since he did not find 'mast cell centers in lymphoid tissue and no cell forms were encountered intermediate between lymphocytes and mast cells" Our observations confirm Bates statement that no intermediate cell forms were found

A notable increase in the number of mast cells is often definitely associated with some condition conducive to increased local protein (to cause transformation of undifferentiated cells into mast cells) such as occurs in hyperemia with increased capillary dilation and leakage of protein and carbohydrate, lymph stasis and/or necrosis of cells or degeneration of collagen Thus the tenet of Caspersson and colleagues that the "presence of high nucleic acid concentrations is essential for every biological protein synthesis' to take place[39] applies to the transformation of mast cell from undifferentiated mesenchymal or other cells Conversely the destruction of mast cells to liberate histamine is very important in the production of hyperemia and increased capillary permeability as a means of concentrating the essential substances for biological protein synthesis in tissues The consecutive steps would be (1) liberation of histamine and heparin (2) hyperemia (3) capillary leakage (4) fibroblastic proliferation with the formation of AMP and (5) mast cell 'invasion" Collagen is also *formed but not in a significant quantity until increased mast cells appear* This was the sequence of the cellular changes observed in the mammary glands of 19 virgin hamsters 43 to 96 days old that received 3 to 29 subpannicular[198] injections of aqueous estrogens (1 4 to 29 mg Lakeside) during periods varying from 1 to 64 days[530] Comparison of consecutive sections *of the mammary gland of the estrogen treated hamsters stained* with toluidine blue by Hotchkiss PAS and Mallory s aniline blue connective tissue methods showed that the mast cells increased during but not after the formation of acid mucopolysaccharides (AMP) and collagen

Jernstrom[480] points out that it is especially noticeable in non infected tumors that pooling or stagnation of lymph in the tissues greatly favors the formation and accumulation of mast cell There is considerable evidence that the so called infiltration of mast cells at sites of collagen degeneration[630] is the result of the transformation of certain undifferentiated

far more significant source of AMP, but they do not have a primary function of storing heparin or other AMP, intracellularly Thus the mast cells merely represent accessory synthesizing and storage cells for AMP, except for special conditions The greatest quantity of AMP is produced by fibroblasts A significant function of mast cells is to synthesize and store this form of polysaccharide in tissues and organs in which AMP cannot be stored extracellularly in an adequate quantity as it is in the ground substance of most of the loose connective tissues For example, mast cells are most abundant in the connective tissues of those organs that have comparatively little ground substance the connective tissue stroma of actively and predominantly, although intermittently functional mucopolysaccharide (MPS) secreting glands or the sublingual salivary gland and Brunner's glands in the duodenum[5,7,8] and synovial membranes [0]

In addition to fibroblasts, some epithelial cells such as the goblet cells of the intestinal mucosa and of certain other epithelial tissues are capable of synthesizing AMP These simple epithelial cells in contrast with the mucin secreting alveolar cells such as in the sublingual salivary gland, have available quantities of AMP in the ground substance AMP secreting epithelial cells significantly occur as a single layer immediately adjacent to relatively considerable quantities of AMP produced by fibroblasts Mast cells have the distinctive function of storing as well as synthesizing AMP Fibroblasts and epithelial cells however are able to synthesize various types of AMP but with the exception of goblet cells they commonly do not function as AMP storage cells Thus the AMP produced by fibroblasts and epithelial cells is temporarily stored chiefly as intercellular ground substance

The work of Bensley[71] supports the tenet that fibroblasts are the chief source of metachromatically staining ground substance in the pancreas of guinea pigs in which the pancreatic duct had been ligated for 10 to 14 days and in the slightly edematous mucous stroma of the human uterus after the period of menstruous repair The ground substance gave no appreciable metachromasia with toluidine blue in either case until fibroblastic proliferation became obvious However neither the presence nor the absence of mast cells in the pancreas or uterus is recorded

The absence of mast cells in blood and lymph and their presence in connective tissues suggest that their origin and disintegration occur in the connective tissues Thus mast cells are unlike lymphocytes in being essentially fixed cells but are similar to plasmacytes because they are not normally carried in the blood stream For instance Ewing [9] states that he has never seen more than single examples of mast cells in blood smears Jordan[40] noted the absence of mast cells in blood although he observed

FUNCTIONAL RELATIONS

The mast cell appears to be a synthesizing and storage cell for fundamental forms of (AMP) Unlike small lymphocytes and eosinophils, the mast cell is a relatively fixed cell and cannot transport its products effectively Tissue mast cells of vertebrates are morphologically identical with trephocytes in invertebrates,[606] and are recognized as trephocytes in most vertebrates [60]

Mast cells appear to be related to multiple metabolic processes, many of which may be attributed primarily to the presence of certain components, chiefly AMP in the mast granules Results of our work and that of several other investigators support the tenet of Asboe Hansen [9, 24] that mast cells play an important part in the formation of hyaluronic acid (HA) However these results also indicate that mast cells have additional essential relations to other processes such as the formation of collagen, heparin, mucin histamine possibly serotonin and dermal pigmentation and, that these cells play a significant part in the detoxication of certain substances The e functions, however, cannot be attributed entirely to mast cells The mast cells are a sort of special accessory adaptation for storing and providing readily available components for various proces es and histamine to increase capillary permeability It has been recently shown that mast cells contain serotonin (5 hydroxytryptamine)[63, 76, 894] which they synthesize from 5 hydroxytryptophan by the action of the enzyme 5 hydroxytryptophan decarboxylase [576]

The trephocytic relations of the mast cell appear to be primarily dependent upon the nature of the substances which it has tored in its mast granules Thus the functions of the mast cell proper (the nucleus and cytoplasm) appear to be limited to (1) the synthesis of the contents of the granules (2) the storage (including ripening processes) of these granules and (3) their release at propitious moments However because of other implication including those of a cytogenic nature any recorded approach to analysis of the functional relations of mast cells leaves much to be desired The problem is further complicated because despite the multiple functions ascribed to them [693] it is not obvious whether or not mast cells are actually essential under other than limited conditions for fibroblasts and certain epithelial cells are al o capable of synthesizing AMP However, it is doubtful whether or not fibroblasts and epithelial cells could obtain the nece ary components for synthesizing an appreciable amount of AMP without the aid of histamine in producing hyperemia and increased capillary permeability Mast cells are by no means the most important or even a proportionally significant general source of AMP when compared with fibroblasts Quantitatively fibroblasts repre ent a

amount of AMP in granules of mast cells represents a very small percentage of the total AMP that are synthesized in normal or in most pathological tissues (3) Mast cells are related to several varied aspects of AMP metabolism such as mucin secretion and the formation of HA which make it more difficult for one to discern the essential function of these cells (4) Mast cells are not primarily associated with the production of a single type of MPS such as mucin while goblet cells apparently obtain AMP chiefly from the ground substance underlying the basement membrane mast cells take over the function of supplying AMP for several mucin secreting glands, such as the sublingual salivary glands (5) The formation of AMP by mast cells is not a continuous or regularly recurrent process rather, it is a single process which is not duplicated as is the case in the formation of collagen in scar tissue

Effects of Nutrition

The mast cells were so named by Ehrlich (1879) because he believed that they were overnourished connective tissue cells and inadvertently he thus directed research efforts along the line of nutritional relations of these cells Some investigators support the idea that mast cells increase under optimal nutritional conditions and decrease under adverse nutritional conditions of the individual However others maintain that the number of mast cells is not altered by either optimal nutrition or by inanition [693] After observing the presence of many mast cells in the fat of fish and in the intestine of well fed salamanders turtles and horned toads and either the paucity or entire absence of these cells in the gut of the last three species following hibernation Michels[693] suggested that there may be a possibility of a functional correlation of mast cells with nutritional conditions Tuma s (1928) work supports this view since he reported that in normally fed rats the mast granules stained readily with Scharlach R Sudan III and ammoniacal silver but that in vitamin deficient rats these methods failed to stain the mast granules [6J3]

The presence of splanchnic basophile cells in the intestinal villi and their tendency to form a distinct layer at the bases of the villi suggested to Hardy and Westbrook (1895) that the mast granule is a center of synthetic activity in respect to the food which passes through the mucosa [693]

Starvation apparently reduces the mast cell population at least in some animals or under certain conditions however the available data are scanty and mostly incomplete particularly because many of the observations were made under variable conditions and were sometimes limited to a single tissue or structure Westbrook (189o) found that animals under starvation had a greatly reduced number of granules in the mast cells

a high content of these cells in the loose tissues along the walls of the smaller blood vessels and in bone marrow Contrarily, Nagayo[731] lists bone marrow as one of the internal organs in which mast cells do not occur

The distribution and number of mast cells indicate that the mast granules may not be a specific form of AMP, but rather that they contain an essential, although inactive basic form of AMP which is released from the granules to become a specific polysaccharide such as heparin or hyaluronic acid or they may supply components for the secretion of other MPS, such as mucinogen The number and distribution of mast cells has further significance from the standpoint of the concentration of AMP because there is a need for synthesis and extensive storage, as well as ready availability, of AMP in several tissues that either do not contain sufficient amounts of ground substance or do not contain many fibroblasts *Thus, there is normally a concentration of mast cells in such tissues as the* mesentery and synovial membrane Although the mast cell is not the only, or even a significantly voluminous source of AMP it is important in the metabolism of AMP This is because it is a type of synthesizing and storage cell that is capable of unloading its preformed contents almost instantly, in contrast with fibroblasts and epithelial cells which commonly produce a small continuous supply of AMP over longer periods of time

Apparently, the mast cell has the ability to release simultaneously both heparin and histamine from an inactive to an active state[854, 1085] Since heparin may exert an inhibiting or controlling effect on the release, or action of histamine[416] the combination of heparin with histamine in inactive form appears to be an unusually effective means of storing these physiologically potent substances The distribution of mast cells in the sublingual salivary gland[5, 7] and Brunner s glands[7, 5, 8] where mucigenesis is very active at irregular intervals and fibroblasts are not numerous, supports this idea

The large number of mast cells in the mesentery of most mammals also indicates that the mast cell stores AMP However the relation of mast cells to the small mesenteric tributaries of the hepatic portal vein and subsequently *to detoxication processes in the liver may be another reason* for the large number of these cells in the intestinal mesentery and great omentum

The significance of mast cells in AMP metabolism is not widely considered by investigators for at least five reasons (1) Mast cells are not recognizable in most routine histological preparations because the granules are soluble in most of the usual fixatives employed and/or are not stained by the usual staining methods (2) Fibroblasts probably produce a greater volume of AMP and certain of its more specialized derivatives than do the epithelial and mast cells under normal conditions Indeed the

is suggested This militates against the idea that their form and size is maintained by interfaces as in an emulsion

Julen Snellman and Sylven (1949, 1950) believe that the mature mast granule probably has an orthochromatically staining protein nucleus or core covered by a metachromatically staining substance which is presumed to be 'well esterified and active heparin'[8] Berg (1951) states that the mast granule appears to consist chiefly of lipid strongly bound to protein[8] Hedbom and Snellman[414] analyzed washed large mast cell granules (presumably Type II of Riley)[8, 7] that were isolated from the hepatic capsules of fresh frozen bovine livers The average values of the total amount of the acid soluble, lipid and protein fractions which they found expressed in per cent of the dry weight,' were nitrogen, 13 0, phosphorus 1 40 sulfur 0 39 proteins and nucleoproteins 72 0 and lipids 23 0 Studer[990] points out that mast granules are not affected by desoxyribonuclease or ribonuclease, which indicates the presence of little or no nucleic acids

There has been a marked tendency for a number of investigators to ascribe a single function to mast cell granules, while others regard these granules as being capable of playing important parts in supplying components for several physiologically active substances such as heparin histamine serotonin and HA Certain investigators believe that the substances are stored in a free, or nearly free state within the containing granule and are more or less mechanically liberated by lytic agents or by autolysis Others believe that the substance (or substances) is stored within the granule in a bound or otherwise inactive state and requires activation, as well as lysis of the granule Equally significant may be the combination or coexistence with physiologically active perhaps even antagonistic substances within a single mast cell The fact that histamine is stored in cytoplasmic granules especially in mitochondria[80, 6, 7] that Berg (1951) claims the mast granule is a lipid strongly bound to protein[855] and that the nature of the enzyme content of the granule in many species is known probably led Riley[8] to regard the mast cell granule as a heparin containing mitochondrion If viewed from an experimental point of view such a complicated condition is discouraging to contemplate because of the difficultie in establishing proof

The presence of AMP in the granules of mast cells has been fairly definitely established by various procedures and is generally conceded to be an essential component for the synthesis of the active or definitive substances Mast cells are known to contain sulfur but whether the mast granules contain an appreciable amount of this element apparently has not been determined In 2 month old brown Leghorn chickens inoculated with T lyophilized Rous sarcoma virus intramuscularly and injected sys

Starvation increased the number of mast cells in animals, according to Sampanow (1908), whereas Nakejimo (1928) and Nagayo (1928) reported that inaniton caused an increase of up to 20 3 per cent in the numbers of mast cells in the tissues and peritoneal fluid in rats[693] Ballowitz (1891) reported that bats (Vesperugo), which had been previously well fed, showed no "significant change in the number volume or distribution of tissue mast cells' when 'kept without food from October to April'[693] Westphal (1880) reported that people who were dying of emaciating diseases had an unaltered ratio of mast cells[693]

Certain investigators have shown that feeding frogs which had been starved for a long time caused a marked numerical increase in tissue mast cells[693] Nakajima (1928) and Nagayo (1928) reported that when rats were fed on cow s milk the peritoneal mast cell count rose from the normal (3 5 per cent) to 7 6 per cent[693]

Hibernation may or may not increase the number of mast cells A salamander (Amblystoma), turtles, and horned toads had very few or no mast cells in the gut during or after hibernation, but "in the fall the gut contained many mast cells'[693] Hirma and Suomalainen[404] found more mast cells in hibernating hedgehogs than in active animals while Peter[693] reported that mast cells were more numerous in the hedgehog after hibernation and in early spring than during the summer or fall

Nature of Mast Cell Granules

It is almost impossible to make a distinction between the activities of the mast cell as a whole and the physiological activities and relations of its granules The characteristically basophilic usually metachromatically or periodic *acid Schiff (PAS) positively staining cytoplasmic granules* appear to be directly related to the only extracellular activities ascribed to the mast cell However freed intact mast granules apparently are inert until the contained substances are released by dialysis or lysis Koksal[4] found that intact mast granules isolated from subcutaneous fascia' of mice gave the usual metachromatic reaction with toluidine blue but had no coagulant effect on mouse blood The extract which was obtained from the isolated mast granules by the extraction method of Wilander[1004] was strongly anticoagulant and it stained metachromatically with toluidine blue

The question of the structure of mast cell granules has not been satisfactorily answered Apparently since mature granules are fairly uniform the general size and spheroidal form is controlled by certain chemical and/or physical agents perhaps with the aid of a sort of delimiting membrane or interface[374] Because these granules retain their spheroidal form for some time after the rupture of the mast cell, the presence of a membrane

content[7 1] and that Benditt and co workers[63] found the ratio of eosinophils to mast cells to be 1 to 200 in certain areas in rats

Mast cells of dog hamster, man mouse and hairless mouse contain the following substances in sufficient quantities to give a good or strong reaction to the various tests employed thermostabile peroxidase cytochrome oxidase, alkaline phosphatase acid phosphatase,[09 745] lipase, lipids (probably phospholipids) Sudan black positive granules[709] disulfide,[44] sulfur-[3] and, probably, —SH but certain investigators did not find lipoid in mast granules of rats[74 1108] However the most significant trephocytic contribution of these cells to somatic growth as such is indirect—for, normally, mast cells contribute to certain physiological processes by the formation of AMP[1 8] they produce heparin or precursors of heparin,[590 850 854 996 1085 1091] histamine[4 69 80 1045 108] serotonin (5 hydroxytryptamine)[576] and HA[0 4] Nevertheless, the possibility that mast cells may contain histamine or its precursors which supply added amounts of protein to tissue fluids by increasing the diameter and permeability of capillaries,[60 200 390 391 407 41 0 1084] is one of the various possibilities that should not be overlooked The wide distribution of mast cells in vertebrates and various structures of single individuals has resulted in a great amount of literature on the relations and functions of these cells[131 606 691 990]

The granules of mast cells are not pure AMP because histamine (possibly bound with heparin), several enzymes (possibly serotonin) and other substances have been found in them However, the enzymes and other substances are not considered to be primary or characteristic components of mast granules Several enzymes which occur in the granules of mast cell such as cytochrome oxidase[3] peroxidase lipase[708] and alkaline phosphatase[745] are not characteristic of mast cells but are commonly found in various cells

Reported variations in the presence or absence of several substances in mast cell granules have not been consistent and may indicate either that there are differences in the content of the substances in mast granules or that there is contamination or adsorption to granules of some of these substances Compton[181] states that the majority of mast cells treated with Sudan black B for 7 minutes do not stain however many mast cells near fat cells contain positive granules For example the granules of mast cells from the human stomach contain lipoids[790] whereas mast cells in the connective tissue of the rat do not contain stainable lipoidal material or fatty substance[74 1108] Granules of mast cells in man dog and hamster contain more sudanophile substance than those of the rat or mouse[09] However Tuma (1928) reported that the mast granules of normally fed rats were stainable with Sudan III, Scharlach P and ammoniacal silver

temically with sulfate labeled with radioactive sulfur (S^{35}), the mast cells, as well as the normally metachromatic ground substance and metachromatic cytoplasm of the tumor cells, incorporated S^{35}. Incidentally, comparatively few of the investigators manifest any interest in the significance of or the methods by which the mast cell synthesizes and/or stores AMP; most of them are interested in the liberation and/or effects of the AMP products, particularly heparin and HA. Thus it is gratifying to note that von Marbet and Winterstein[1065] direct attention to the fact that because of their great physiological and therapeutic importance, the MPS are being investigated on a much broader scale and much more intensively in the last few years. The result is that heretofore unknown pharmacodynamic properties of AMP are being described and a much wider field of relations has been ascribed to these combinations of MPS which have been known for years. For example, the heparins, chondroitin sulfuric acid, HA and connective tissue ground substances are known to play variously important parts in transportation and water balance in cellular metabolism.

The controversial literature on the ubiquitous mast cell, which includes widely diversified ideas concerning the origin, development, morphology and functions of this cell, as well as the identity of its components and stored contents and the means by which these substances are stored and released, suggests that the relations of the mast cell to enzymic activity may be somewhat complicated. Although Sehrt (1927) recognized the presence of oxidase in mast cells,[693] apparently little work has been done on enzymatic activity involving mast cells until very recently.

Gomori,[35] using chloroacetate of alpha naphthol or naphthol AS chloroacetate as the substrate, demonstrated the activity of an enzyme present in mast cells which reacted strongly and preferentially with mast cells and less rapidly with myeloid elements in paraffin sections of acetone or neutral formalin fixed tissues from dog, man, mouse, rabbit and rat. Although he did not identify this enzyme, he is certain that it was not a cholinesterase.

Benditt,[61] using the chloroacetyl ester of 2 OH 3 napthoic acid anilide (CANAS) as a histochemical substrate, demonstrated the presence of an enzyme in mast cells obtained from rats which had certain properties that resembled those of chymotrypsin. When he compared the activity of the mast cell enzyme on the substrate with that of crystalline chymotrypsin, he found that 6.5 mm³ of the mast cells were equivalent to about 80 μg of the chymotrypsin. The mast cell enzyme was derived from purified mast cells isolated from the peritoneal cavity of rats and from a homogenate of rat feet skin. In connection with these findings it is interesting to note that the skin and subcutaneous fascia from the feet of rats have a very dense mast cell population[63, 71] and a very high (61.0 μg/g) histamine

content[7, 1] and that Benditt and co workers[63] found the ratio of eosinophils to mast cells to be 1 to 200 in certain areas in rats

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whereas Sehrt (1927) attributed the positive oxidase reaction of mast cell granules to 'their lipoid content which has the power to fix oxygen'[693] Several other investigators have reported that they were unable to obtain positive reactions of the granules with various lipoidal or fat reagents, Hammer (1912) found that lipoid solvents had little or no effect on the granules[693]

FIXATION AND STAINING

Metachromasia which is a common reaction of mast granules with certain cationic aniline dyes,[91, 1001] and PAS reactions are briefly discussed in the following section The usual methods of fixing and staining animal tissues do not consistently demonstrate mast cell granules[496, 970] and similarly blood smears stained with toluidine blue give a higher mast leukocyte count than when stained by the May Gruenwald Giemsa method[970] However for several decades at certain laboratories (notably at the Zoological Laboratory University of Pennsylvania under the direction of C E McClung), test tube or filter paper ring spread preparations were made of omentum as routine class work to demonstrate mast cells However this method was of little aid in preparing other tissues for the study of mast granules

Routine Fixation and Staining

It is generally known that routine fixing and staining methods are not reliable when used to demonstrate mast cells[500, 693, 864] Many (probably most) of the investigators avoid the use of aqueous fixing solutions in preparing mast cells especially mammalian tissues[51, 496] or mammalian and lower vertebrate tissues[693] Some including Bolton (1933) recommend Helly s fluid or bichloride of mercury in aqueous solution[693] However Carnoy's fluid probably because of its acidity preserves the granules poorly[51] *Although investigators have been warned by Jorpe*[500] *Michels*[693] and many others that mast cell granules are soluble in most aqueous solutions surprisingly few authors or readers take this axiom seriously Several fixing and staining techniques have been devised to prevent the loss of mast granule substance Some investigators believe that lead is essential for this purpose in fixing fluids for mast cells A highly recommended mast cell fixative contains 1 per cent lead subacetate and 0 5 per cent acetic acid in 50 per cent alcohol[7, 1] Asboe Hansen[18] and Iverson[469] favor a 4 per cent solution of lead subacetate and deplore the use of alcoholic solutions We[6, 5, 7, 5, 8] have found that mast cell granules in mammalian tissues fixed in a solution of bichloride of mercury in alcohol stain well and do not dissolve when deparaffinized sections are left in water or salt solutions for 48 hours

We have successfully used sublimate alcohol (7 1 per cent mercuric chloride in 70 to 75 per cent ethanol), to fix mammalian tissues in order to study mast cells with toluidine blue O, and other methods for several years This fixative is rapidly prepared as follows put 280 g of granular or powdered sublimate in a clear glass bottle with 2890 0 ml of 95 per cent ethyl alcohol and shake until it is dissolved Add 1030 0 ml of water Tissues in ample volume of the solution fix thoroughly in 12 to 24 hours

Metachromasia

Ehrlich introduced the term "metachromasia" in 1879 to designate the staining reaction of a structure in a tone differing from that possessed by the dye[693] Szirmai[1004] points out that metachromasia is a reaction of basic dye molecules bound to negatively charged macromolecules (sulfate phosphate or carboxyl groups) Several investigators believe that the metachromatic color of mast cells is stable in alcohol and is thereby unlike that of amyloid and to a less extent other metachromatically staining substances, for example, mucous materials and cartilage[91*]

There is considerable albeit not always clear evidence which indicates that metachromatically staining mast granules usually occur in the older (Type II of Riley[8]) mast cells and that their granules especially in the rat contain only the higher sulfates of heparin the di and trisulfates, as shown by Jorpes and colleagues (1948) The younger mast cells (Type I of Riley) contain only the mono sulfate of heparin and consequently stain PAS positively[8] or orthochromatically but do not stain metachromatically Schubert and Hamerman[91] in their exhaustive study of metachromasia, extend and elaborate Ehrlich's early basic and Szirmai's[1004] contemporary definition to include not only the metachromatic coloration of tissues and structures but also certain systems in solution Metachromatically staining anionic structures are called chromatropes since they cause the color changes in cationic aniline dyes known as metachromasia Schubert and Hamerman caution that since there is much controversy over the distinction between true and false metachromasia the reliability of various metachromatic dyes the chemical nature of the chromotrope presence of inhibitory factors in the tissues and other conditions metachromatic staining is one of the more treacherous histochemical techniques Several non chromotrophic substances are recognized as inhibitors of metachromasia Thus salt cations which may prevent metachromatic staining of mast granules especially if the dye is in low concentration and certain proteins notably gelatin and albumin in staining hyaluronate inhibit metachromasia in tissues[91] Iversen[469] states that in normal animals made thyrotoxic by administration of thyroxin the connective tissue ground substance loses most to all of its capacity to stain metachromati

cally with toluidine blue, and only a few mast cells may be seen Since a number of investigators, some of whose works are cited in this section, give extensive discussions on the chemistry, microtechnique and significance of metachromasia we shall comment only briefly on some of the controversial points of this subject

Pearse [70] using aqueous solutions of toluidine blue and mounting fresh tissue in glycerine jelly or corn syrup recognizes two types of metachromasia (1) beta (violet color) can be caused by highly polymerized carbohydrate or phosphate containing compounds , and (2) gamma (red color), probably due to sulfate esters He states that AMP usually give gamma metachromasia and that polymerized compounds containing carboxyl or phosphoryl groupings and occasionally 'nucleic acids in paraffine give beta metachromasia It is also pointed out that the metachromasia of toluidine blue does not give a clear differentiation between the sulfated MPS and hyaluronate [686]

Kramer and Windrum [61] show that sulfation (fuming sulphuric acid acetic anhydride ether mixture) does not impair the subsequent metachromatic staining of mast cell granules but that certain other PAS positive structures such as basement membranes show intense gamma (red) metachromasia Wislocki and co workers [1109] found that mast cell granules of man monkey and swine which were strongly PAS positive stained as intensely metachromatically with toluidine blue as did the granules of the rat which were more faintly PAS positive

Hebdom and Snellman [114] found that the mast granules from homogenates of ox liver capsules suspended in 0 25 to 0 88 M sucrose or a salt solution for some time when supravitally stained with azure A were an orthochromatic blue while those granules treated for varying short periods also stained orthochromatically but were surrounded by metachromatic halos of varying densities We also obtained metachromatic halos by subjecting sectioned tissues which had been fixed in sublimate alcohol to long exposures in a wide range of molar solutions of NaCl and staining with toluidine blue These results strongly indicate that the strength of the salt solutions used by some investigators in extracting heparin like substances from mast cells probably accounts for their variable findings and conclusions [970, 995]

Unfortunately as several investigators [181, 686, 693, 790] point out the metachromatic staining of granules of mast cells fails to provide adequate information on the chemical nature of these structures because the significance of metachromasia has not been definitely established Lison (1936) attributes metachromasia to the presence of esters of sulfuric acid having a high molecular weight whereas Kelley and Miller think that all substances that have a high molecular weight and an acidic function are

metachromatic[790] Weiss[1079] attributes metachromasia in mast granules to the sulfate groups which are present in heparin However, since HA is a sulfate free carbohydrate,[99] Wislocki and co workers[1109] point out that the sulfate linkage is not necessary because HA stains metachromatically Furthermore it has repeatedly been shown that treating mast cell granules with hyaluronidase does not abolish the metachromasia in them[151 686 996] Since hyaluronidase merely liberates the disaccharide in HA, and any further degradation of this acid is "due to other enzymes" [6] hyaluronidase could hardly be expected consistently to efface metachromasia in either mast granules or in HA

Gatenby and Painter[336] suggest that it would appear that in thionin stained tissues the change of mucin metachromasia to orthochromasia by alcohol and back to red by water is one 'by which an amino group becomes freed from its combination with the mineral acid of the salt,' possibly as Holmes (1926) has shown where 'an addition product may result in which the pentavalent nitrogen becomes trivalent with corresponding change in color In the case of thionin the acid is suposed to change its connection to the nitrogen which unties the two benzene rings However a number of investigators take exception to this idea[66]

Another explanation of metachromasia is that it may be due to tautomerism[790] because the change does not involve great alterations in chemical structures[336] Schubert and Hamerman[91] indicate that any explanation of metachromasia must include consideration of the shapes of polyelectrolyte molecules in solution and the significance of absorption bands of solutions of dyes as well as the formation of water insoluble compounds Szirmai[1004] suggests that control of the pH and salt concentration affects the intensity of the metachromasia which can be used to obtain further information on the chemical nature of metachromatically staining ground substance in the cock s comb The strong toluidine blue or thionine metachromasia which Feyrter (1936) observed in the sheath of myelinated nerves in frozen sections has been ascribed to HA by Altschuler and Angevine[6] because they found it to be reversible by using hyaluronidase on paraffin sections[790] Michaelis (1947) discovered that the presence of carboxyl groups causes metachromatic effects in highly polymerized carbohydrates and he also believes that metachromatic effects occur in high polymers of metaphosphate in yeast cells[790]

The interpretation of metachromasia is further complicated by microtechnical problems which in routine procedures seem too trivial to receive any consideration since they involve the effects of alcohol and/or water on determining whether a tissue stains orthochromatically or metachromatically In addition to the orthochromasia histochemically there are two varieties or types of metachromasia that may be obtained with

toluidine blue or other dyes. Michaelis shows that the absorption spectrum of toluidine blue has three bands (1) alpha (monomeric, blue in color, orthochromatic), (2) beta (dimeric, violet in color, metachromatic) and (3) gamma (polymeric red in color metachromatic). He also found that changing the stained sectioned tissues from water to alcohol caused the return of the blue (monomeric) color which Pearse[790] believes is due to the presence of both alcohol and water. Thus, the usual "up grading" through alcohols to xylene would cause most of the polysaccharide containing tissues in toluidine blue stained sections to stain orthochromatically (blue). Just how the combination of alcohol and water converts metachromasia to orthochromasia is not understood but it has been observed that breathing upon metachromatic alcohol wet sections will supply enough water to cause them to turn blue instantly. Properly fixed and sectioned tissues that contain AMP, retain the metachromasia if the alcohol series is avoided by blotting and passing them into xylene.[90]

Mast cell granules cultivated *in vitro* give various tinctorial reactions with toluidine blue that range from negative to an intense reddish purple color[768] while HA stains metachromatically.[393, 693] Like mast cell granule probably because of the AMP it may also stain positively with PAS,[49] as explained by Lillie.[608] Likewise, connective tissue ground substance stains variably.[18, 4, 795]

Compton[181] after reviewing the literature on the significance of metachromasia of mast cells concludes that with our present information about the only safe interpretation which can be made concerning metachromasia of mast cell granules is that they probably but not certainly, contain a compound with high molecular weight and acidic function. The various explanations given for the metachromatic and PAS positive staining capacities of such AMP structures and substances as (1) mast cell granules (2) ground substance of connective tissue and (3) HA, are discussed at considerable length in the literature.[181, 561, 56, 618, 693, 7, 4, 768, 790, 795, 88, 91]

PAS Staining Reactions

The PAS is a general histochemical staining method designed to visualize polysaccharides in tissues for microscopical identification of the contained carbohydrates. Since the PAS method stains only the polysaccharides an excellent triple method described by Himes and Moriber[433] is gaining favor. This method differentially stains deoxyribonucleic acid (DNA) polysaccharides and proteins and thus greatly facilitates the identification of histological and cytological structures other than those which stain PAS positively. The Hotchkiss (1946) method with variations is the most widely used, although McManus independently presented the same prin

ciple in the same year[44] Polysaccharides commonly stain a violet fuchsin color dependent upon the chemical nature and other factors in the polysaccharide and upon variations in the technique including the counterstain used Glycogen mucin muco protein and presumably hyaluronic acid and chitin' may be stained by this method[347] Others find that HA is strongly PAS positive[48] We have found that the basement membrane of various epithelial tissues stains sharply by the PAS method Other investigators also report that basement membranes are PAS positive[4 7 1109]

The staining reaction of granules of mast cells following treatment with the PAS reaction has been considered by numerous authors but has not provided a specific answer to the chemical composition of the granules Meyer[696] believes that the PAS stain does not permit distinction between the various components of the ground substances and from this he deduces that staining reactions would be expected to vary, since HA from various sources vary widely in their physical properties from that of umbilical cord It has been found that differences in reactions to the PAS method occur (1) among mast granules within the same individual and tissue[181 7 609 90] (2) in mast cells of different species[608 1109] and (3) in granules of a single mast cell[469 68] Such variations would be anticipated if the granules of mast cells are a basic form of AMP that can be utilized in tissue fluids and by other cells in multiple aspects of AMP metabolism

The PAS method has been considered to be in part, the cause of some of these discrepancies because slight variations such as water soluble and water insoluble methods are used to identify different kinds of carbohydrates[191 347 90] Also slight variations in application could give diverse results Individual variations in the use of the PAS method do occur Pearse[790] states that it must be emphasized that differences in the PAS reactions of given structures reported by different workers are as much due to variation in technique as to variation in the structures themselves For example Compton[191] found that mast cells were negative at 15 minutes in the Schiff reagent but that many but not all mast cells were positive when left for 2½ hours

Another cause of differences in the reaction of the granules to PAS may be because different granules contain AMP in varying degrees of polymerization Pearce[90] points out that the concentration and degree of polymerization of a substance is an important factor in controlling the color reaction

Differences in metachromasia of the ground substance in connective tissues have also been attributed to differences in the degree of polymerization[790] and to variations in methods of preparation of the tissues especially in fixation and staining[8 9] Also polysaccharides other than those occurring in the granules of mast cells differ in staining reactions Meyer[696]

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states that HA from various sources likewise vary in their staining reactions and also in their physical properties He suggests that the fraction of HA from the cornea may be chemically distinct from that from other sources

Variations in response to PAS technique have additional significance when time relations are considered for the incorporation of sulfuric acid into polysaccharides Meyer[696] states that the sulfate is rapidly fixed in growing connective tissue but that the stage at which the carbohydrate chains are sulfated is not known It has not been established whether polymerization or sulfation occurs first Similar possible variations in the sulfation of the AMP in granules of mast cells would account for difference in reaction of granules in mast cells to the PAS technique Jorpes and collaborators (1948) believe that while heparin monosulfate would react to periodic acid the higher esters would not [90] Jorpes, Werner and Aberg (1948) also found that heparinocytes' of young rats were PAS positive because of the presence of monosulfuric heparin [181]

C Smith has shown that the age of mast cells has been considered as a possible reason for differences in the reaction of the mast granules of mice to the PAS technique [608] Age of the cell is also a factor in determining the metachromatic staining reaction of granules of mast cells Michels[693] states that immature granules give an orthochromatic basophilia instead of a metachromatic reaction Compton[181] also suggests that differences in staining with PAS technique might be due to different functional states within the cell as well as to other factors such as variations in dissolution of granules during staining in spreads of different thicknesses

FACTORS AFFECTING MAST CELL POPULATION

Mast cells have not been considered by many of the investigators working in the broader fields of experimental histology Much of this neglect *in the study of mast cells is probably due to the inadequacy of the methods* employed and as a result there has been little interest in the pharmacophysiological significance of mast cells until the past few years

Factors Increasing the Number

A number of limitations are imposed upon the investigator who attempts to establish quantitative changes in the mast cell population The immediate progenitor of the mast cell is not definitely established and it is almost impossible to identify the early or pregranular stages Some of the granular stages stain metachromatically and others stain PAS positively One of the more accurate methods for counting mast cells, when it is applicable is air dried tissue spreads fixed with absolute methanol and stained in Giemsa's mixture buffered at pH 5 0 [63]

Many investigators relate some, if not all, of the allergic and anaphylactic reactions to mast cells but it is difficult to determine whether the mast cell is the responsible, or etiological agent, or is merely a result of the reaction These and other points which have previously been mentioned suggest some of the difficulties encountered in attempting to quantify mast cell populations

It is common knowledge that, in many instances administration of any of several agents which deplete hematopoietic cells[89] and lymphoid tissues and produce lymphocytopenia is followed by an increase in the number of mast cells, whereas certain conditions which promote a local increase in lymphocytes plasmacytes, monocytes and eosinophils are also conducive to the formation of mast cells It is too early in the study of mast cell relations to make a definite statement but in general it appears that those agents which deplete lymphoid tissue lymphocytes and eosinophils contribute to the general or delimited increase in the number of mast cells Cortisone however appears to be an exception to this general rule

IRRADIATION

Mast cells are resistant to most forms of irradiation and radioactivity [89, 79] Michels [69a] using a dose of 400 r of x rays on the omentum found that mast cells are the most radioresistant of any of the connective or other cells in the omentum A single total body x irradiation (995 r) of 22 male and 33 female hamsters 82 to 101 days old failed to produce an increase in mast cells or PAS positive material in the adrenal glands kidneys liver mesentery ovaries, pancreas, salivary glands, spleen, uterus or testes [53] Nevertheless irradiations may be responsible for a selectively stimulating effect on mastocytogenesis in certain structures in hamsters and other animals

Lymph nodes Lymphoid tissues are normally about the most sensitive of all tissues except possibly gonadal and erythropoietic to lethal types of irradiation The scanty information available to us indicates that almost all if not all irradiations which are sufficient to destroy circulating lymphocytes and to deplete lymphoid tissue increase the number of mast cells However it is not clear whether this increase in mast cells is a direct result of the irradiation or whether it is a response to the release of substances by other x ray injured cells It appears probable that the mast cells utilize a significant amount of the cellular debris in the synthesis of the MPS stored in the mast granules This idea is supported by the observation that in chronic gamma irradiations extending over a period of several months there was a progressive increase in mast cells while the lymphoid cells speedily decreased [89]

Thymus Mast cells are usually not considered in the voluminous litera

ture on the effects of irradiation on the thymus Murray,[9] after discussing the depleting effects of a wide variety of forms and methods of irradiation on the thymus of various animals, states that the number of mast cells appears to be increased following the internal administration of nearly all agents that cause thymic depletion

The thymus was not affected in mice subjected to 500 rep of external beta irradiation which killed about 20 per cent of them[89] However, Spargo and co workers (1951) have shown that gamma rays in daily doses of 1 1 r for 10 months 4 4 r for 8 months or 8 8 r for 6 months depleted the lymphocytes but there was a striking increase in the number of mast cells in the thymi of all of the irradiated mice[89] A single total body exposure of 995 r of x irradiation actually, as well as relatively, increased the number of mast cells in the thymus[5,6] of 17 out of 25 hamsters in which the thymus was in the inversion stage (greater decrease of lymphocytes in the cortex with relative increase in the medulla) and in 12 out of 23 in which the thymus was in the advanced stage (lymphocytes depleted in both cortex and medulla) of involution (fig 17)[6] The PAS positive fibrous tissue and collagen increased during this depletion—a change which suggests that the mast cells might be a cause, or source of AMP[09]

Lehner believed that the reduction in the size of the thymus and the gradual depletion of its lymphoid tissue during the process of normal thymic involution should be attributed chiefly to heteroplastic differentiation of the thymocytes into mast cells[693] If this explanation could be substantiated it would be a neat way to account for the increase in thymic mast cells during age involution However, the extreme sensitivity of lymphocytes especially thymocytes to a number of unfavorable conditions would preclude the application of this explanation of thymic mast cell increase to most instances of accidental involution especially to those resulting from the widely known effects of irradiation

Thyroid and parathyroid glands Mast cells increased in the thyroid glands in 10 of the 21 hamsters (12 males and 9 females) 96 to 300 days old which were exposed to 800 to 995 r total body x irradiation and sacrificed 5 to 30 days after irradiation The increase in the number of mast cells was actual in 3 of these hamsters 2 of which had hypertrophied thyroids but only relative in 7 The parathyroid glands in the 2 animals which had hypertrophied thyroid glands were slightly hypertrophied and showed a relative increase in mast cells[1]

Spleen Many investigators have shown that various types of irradiation reduce the size of the spleen but Senn (1903) was probably the first to observe and describe the decreased size of the spleen following x irradiation[786] of a patient suffering from splenomedullary leukemia It is probably safe to assume that mast cells are increased in the spleen by various if

not all, radioactive substances when the injected dosage is sufficiently high and the life of the animal sufficiently prolonged However, the sensitivity of lymphocytes and plasmacytes to irradiation suggests that it would be incorrect to attribute the increase in number of mast cells in lymphoid organs subsequent to irradiation to heteroplastic differentiation of lymphocytic cells into mast cells as is commonly believed to be an important source of these mast cells[693] in normal animals

Intraperitoneal injection of strontium 89 in do es near that found to kill 50 per cent of the mice in 30 days ranging from 0 5 μc/g to 6 8 μc/g (7 0 μc/g LD $_{0}$, 30 days), produced an increase in the number of mast cells in the spleen of animals killed at late intervals (presumably around the 4th week) [9] The red pulp of the spleen contained many mast cells after 60 days in another series of an ABC strain of mice that received only 0 5 μc/g [9] Mast cells were increased in the spleen of rats intraperitoneally injected with strontium 89 in doses ranging from 2 0 to 10 0 μc in the animals that survived from 7 days to 6 months [7 9]

The spleen of mice that received daily doses of 4 4 r x rays up to 16 months and of those that received 8 8 r daily up to 6 months all showed a progressive increa e in the number of mast cells which in the 8 8 r group reached 4 5 times that of control mice [89]

Skin Mast cells are numerous in the connective tissue of the skin However most descriptions of the general effects of irradiation on the ti sues and organs especially in connection with irradiation from an external source do not include a comprehensive discussion of the effects on mast cells of the skin Earlier investigators observed an increase in the number of subcutaneous mast cells following total body irradiation of laboratory animals [693]

Numerous investigators point out that there is a notable increase in the number of blood vessels and dilation of the capillaries and lymph ducts in radiation produced cutaneous erythema [786] One would expect erythema producing irradiation to cause mast cells to rupture and relea e histamine for the following reasons (1) Released histamine can cause dilation of these blood and lymph vessels [1037] (2) Most of the histamine of the skin is derived by release from dermal mast cells (90 per cent is released by compound 48/80) [97] (3) Irradiation releases histamine [416] This idea is supported in part by Snider s[956] and Snider and Raper s[9] observations that 8 hours after total surface exposure of mice to 2500 and 5000 rep doses of beta rays the mast cells in the kin were beginning to break down [9 6] and that there was a conspicuous breakdown of mast cells in the skin (effects of 2500 5000 and 8800 rep differed only in degree of damage) [957] Three weeks after irradiation Snider and Raper found only a few arterioles and venules which had been obliterated and they rarely

observed mast cells or dilated lymphatics in the skin of these mice One week later (4 weeks postirradiation) they observed 'mast cells in normal numbers' with occasional dilated capillaries and lymphatics in the considerably edematous, abnormal areas of the derma The "mast cells were usually present in normal numbers" and were accompanied by many lymphatics and small, dilated blood vessels and subepidermal degeneration of collagen in these mice 3 months after irradiation Although these investigators show that the dermal mast cells have returned to about normal 3 weeks to 3 months after total surface beta irradiation, it is not clearly shown that external beta irradiation should be considered an agent to increase dermal mast cells

Ultraviolet light was found by von Mollendorf (1928) to produce an increase in the number of subcutaneous mast cells in rats[693] It has also been reported that x rays caused an increase in subcutaneous mast cells within 5 days but that no increase in mast cells was observed until several months after application of tar or arsenic to the skin of rats[693]

Bone Marrow Wallbach, as early as 1929, showed that mast cells increased in bone marrow after a dose of 200 r of x rays[693] Spargo and coworkers (1951) show that in mice external gamma rays in daily doses of 4 4 r for 6 months or 8 8 r for 4 months produced a progressive increase in the number of mast cells in bone marrow[89] This was accompanied by progressive increases in mast cells in the spleen and thymus but 1 1 r daily for 16 months had no effect on the marrow[89] In this connection Jolly s (1924) observation that femoral marrow was very much more resistant than popliteal lymph nodes to x rays in the rabbit[1] is of comparative interest

HORMONES

The debatable relations of hormones to mast cells are discussed in Chapter 5 Histamine In general those hormones which favor conditions for plasmacytogenesis and protein storage such as growth hormones (STH)[150] and estrogens which affect the mammary gland[3, 30, 533] and stratum vasculare of the uterus[30] and those having phlogistic action[9] such as desoxycorticosterone (DCA DO DOC DOCA)[391] increase mastocytogenesis Those hormones which have an antiphlogistic action particularly adrenocorticotropic hormone (ACTH) and cortisone inhibit mastocytogenesis[18] Baker[38] was unable to determine that adrenocortical hormones had any direct action on mast cells in the omentum of the rat He found a slight increase in the number of perivascular mast cells in adrenalectomized rats but attributed this increase to the depletion of omental fat He also found that although subcutaneously administered cortisone suppressed the action of intraperitoneally injected peptone

which causes leukocytes to pass out of mesenteric blood vessels it failed to protect the mast cells against concurrent lysis and release of mast granules Chronic administration of thyroxin depletes the mast cell population and destroys the metachromatic response of connective tissue ground substance to toluidine blue in otherwise normal animals[469]

INCREASED PROTEIN

The relation of protein to increased mastocytogenesis is not well understood, for multiple factors apparently are involved In general a prolonged increase in alimentary or of local protein is conducive to increased numbers of mast cells and yet x irradiation which interferes with protein metabolism, is also conducive to increased mast cells in thymus whereas cortisone, which accelerates protein catabolism[419] depresses the number of mast cells[1 0]

There are a number of observations which indicate that increased protein intake especially in animals which have been subsisting on an inadequate diet is conducive to a general increase in mast cells However it is difficult to determine in most instances whether the increase in mast cells is the result of the added protein in the diet or of certain fractions (vitamins minerals) which may have been introduced with the protein

Michels[693] found that prehibernating horned toad turtles and a salamander (Amblystoma) had many mast cells in the intestine whereas those studied during or after hibernation had few or no mast cells in the intestine Nagayo (1928) and Makajima (1928) reported that feeding cow s milk to adult rats nearly doubled the number of mast cells in the peritoneal fluid—the actual value given was an increase from 3 5 to 7 6 per cent[693] When rats used in inanition experiments were fed cow s milk there was a great increase in the mast cells in the tissues, whereas in the peritoneal fluid the increase reached 20 3 per cent in some animals[693]

Mast cell population is increased locally by increased protein as Peter[798] has shown is the case in certain degenerative changes Mast cells increase as a result of the release of protein (and other substances) by degenerative changes and as others[693] have shown in incomplete stasis of lymph exudate intercellular fluid and in chronic inflammation is apparently contingent upon the presence of undifferentiated mesenchymal cells which may function as stem cells for production of the mast cells Fibroblasts probably meet this requirement more often than reticulocytes or other cells

This conclusion is the result of (1) our[0] study of the disposition of ribonucleic acid (RNA) that is released from cytolysing sarcoma (HS 6) cells during the process of miliary necrosis in 114 hamsters and (2) our[08] finding mast cells absent in both the miliary and large necrotic areas and in

the remaining viable tissue of the tumor in 3 sarcomas (HS 4, HS 5, and HS 6) and 1 carcinoma (HC 2) in a total of 166 tumor bearing hamsters and in spontaneous adenocarcinoma in 20 mice Mast cells were either absent or very scarce in the stroma of all of these tumors, and were not significantly numerous in the connective tissue adjacent to these advanced tumors Other investigators report that "mast cells are usually absent or extremely sparse in deeply infected areas of carcinoma" but the adjoining normal tissues usually have accumulations of these cells[693]

CHEMICAL CARCINOGENS

The chemical carcinogens, most of which belong to the group of chemicals known as polycyclic, aromatic hydrocarbons or to the heterocyclic analogues of these include 3 4 benzpyrene which is present in coal tar[111] Certain of the chemical carcinogens are known to produce a marked increase in the mast cell population when applied percutaneously and, presumably when injected intradermally, subpannicularly intramuscularly or otherwise We have observed a marked increase in dermal mast cells subsequent to painting the interscapular region of hamsters with a solution of 9 10 dimethyl 1 2 benzanthracene in benzene and olive oil However, we have been able to find relatively little material of a clear cut nature on this approach or on the mastocytogenic activity of chemical carcinogens in the literature

Block and Dreifuss (1922) found that the connective tissue was loose edematous and infiltrated with polymorphonuclear leukocytes, lymphocytes plasmacytes and sometimes also mast cells at the beginning of the painting of mice with tar distilled at 300 to 400 C[3 9] Several other *investigators in the early 1920s mention similar findings in mice* and rabbits painted with tar[379] Unfortunately, Yamagiwa and Ichikawa[11 3, 11 4, 11 5] in various papers on their pioneering work on producing cancer by painting the ears of rabbits with tar apparently did not consider the occurrence of mast cells Later however, Ichikawa and Baum (1924) reported that tar increased the subcutaneous mast cells[693] The work of Guldberg[3 9] also shows that painting the ears of rabbits with tar increased mast cells

Direct applications of tar to the skin of mice as reported by Bierich (1922) and various other investigators produced profound increases in the dermal and subdermal mast cells[693] Fabris (1927) subjected mice to an atmosphere containing a rather high concentration of finely pulverized tar at intervals[693] After a period of 3 months he obtained a pronounced generalized dermal and subcutaneous increase in mast cells with a remarkable number of aggregates of these cells in the dermis and subdermal connective tissue The nodular type of aggregates of mast cells and unre

lated plasmacytes were so impressive that Fabris called them "mastocytomas Since large quantities of the tar were deposited on the skin and mucous membranes and caused progressive and extensive loss of hair in these mice[693] it would be very interesting to know if the number of mast cells was increased in the digestive tracts and respiratory system

The stimulating effects of chemical carcinogens on mastocytogenesis apparently are not related directly to the carcinogen but to its ability to produce conditions in the tissues similar to those characteristic of subchronic inflammation

Factors Decreasing Mast Cells

A number of investigators have shown that several agents and conditions may cause a great reduction in the numbers of local or systemic mast cells Michels[693] citing the works of a number of investigators states that mast cell numbers have been markedly reduced by the injection of adrenalin or thyroxin and by various foreign substances injected intraperitoneally into rats Cortisone,[1 0] ACTH and other antiphlogistic agents[9] - and a number of other substances which are usually considered as histamine releasing agents including ammonia stilbamidine[108] ovomucoid[6] and others selectively depress markedly reduce or obliterate mast cells Compound 48/80 is claimed to be nearly specific for lysis of mast cells[1, 8, 7, 104] but as shown by Humphrey (1956) 48/80 has no effect in living platelets[1045] and probably certain other histamine containing cell Most of the so called histamine releasing substances are so very effective in destroying mast cells that in moderate dosage they may cause profound histaminic effects or death

MAST CELLS AND MAST LEUKOCYTES COMPARED

Granules in mature mast cells in tissues are characteristically metachromatic when stained with toluidine blue and otherwise similar in their reactions to granules in mast leukocytes (basophils) in the blood[486, 649] Speirs[970] however states that there is a difference in staining reaction of the granules of these two types of cells Jernstrom[456] believes that the similarity in staining reaction is all that mast cells and mast leukocytes have in common whereas Weiss[1080] makes a distinction which helps to perpetuate a very controversial point He states that the granules of basophils are water soluble while those of mast cells are not Neither our work as explained earlier in this chapter nor that of Michels[693] or of several other investigators supports the idea that mast cell granules are insoluble in water

Tokue[illegible] advocates the view that most of the mast leukocytes are not produced in bone marrow and are not degenerative products of granulo

cytes He believes, also, that if these cells are in any way related to granulocytes it is most probably by derivation from lymphogenous or histogenous cells However, this statement may not hold for the guinea pig, for in this animal the mast leukocytes "resemble true granulocytes"[861]

Mast leukocytes (basophils, basophilic leukocytes, basophil gekornten Myelozyten[911] Blutmastzellen[649]) form only 0 4 to 0 6 per cent of the circulating leukocytes in man, but, at this percentage, the total number of mast leukocytes in the circulating fluid of a man at any one time is approximately 200 million[1] or one mast leukocyte to 120 000 erythrocytes in human blood[746] Ehrlich[7] Martin and Roka[649] Bunting[1] and certain other investigators maintain that mast leukocytes are of myelogenous origin and increase enormously in myelogenous leukemia[649, 663] There is no staining reaction nor morphological difference between mast leukocytes and mast cells[1, 7, 649, 663]

There is an increase in the number of mast leukocytes in the blood in smallpox chicken pox, chronic inflammation of the accessory nasal sinuses and in early Hodgkin s disease especially in lymph node smears[1] These cells (1) irregularly increase in numbers in animals injected with foreign proteins, (2) occur in smallpox pustules and (3) have been supposed to have a detoxifying function[1]

Both mast leukocytes and mast cells apparently contain heparin and polyester sulfuric acid[649] Behrens and Taubert (1952) extracted heparin, or a biologically and physiochemically reacting heparin like substance from isolated mast leukocytes of the horse[649] Martin and Roka[649] obtained a heparin like substance from extracts of leukocytes (dried in acetone) isolated from a patient with mast cell leukemia They state that this appears to be the first time that the probability of the presence of *heparin in human mast leukocytes has been revealed by biological investigation* although as a result of histochemical tests, it had been previously suspected

Mast leukocytes are seldom seen in connective tissue However Webb (1931) showed that egg albumin injected intraperitoneally caused a remarkable increase in the number of these cells in the subcutaneous connective tissue of white rats[693] Fabri (1927) by subjecting rats for 3 months to an atmosphere of finely pulverized tar claims to have obtained the greatest increase in the number of mast leukocytes in loose connective tissue (tissue basophilia) that has been reported in the literature[693]

It is generally believed that mast cells and mast leukocytes are independent cell types[486, 660, 693, 1079] However Weiss[1079] and earlier writers[693] suggest the possibility that mast leukocytes may represent a stage in the life history of connective tissue mast cell or that mast cells may be derived directly from mast leukocytes However, mast leukocytes are claimed

to have a myelogenous origin [7 597 649 693 695 911] The fact that Downey [44] found mature mast leukocytes in imprints of the thymus of rabbits may be taken to indicate, *but does not prove, histogenous relations of mast* cells and mast leukocytes

The mast leukocytes, or basophils which in man comprise about one half of 1 per cent of the leukocytes in the blood[57 96] (1) are considered to be richer in heparin than any other cells in the blood,[366] (2) are much more numerous (33 to 65 per cent) in some of the lower vertebrates,[691] (3) differ in number and size of granules which in basophils of man, are irregular and (4) tend to be elongated and are less numerous in the cytoplasm, in contrast with the round and closely packed granules in mast cells [10 9] Mast leukocytes of human and animal blood 'are oxidase and peroxidase negative," but this is not the case in the guinea pig [10] The human mast leukocyte contains only 1/ to 1/32 as much histamine as the mast cell [366] Other differences observed are that mast cells are larger and have less motility than mast leukocytes,[1103] but are almost never seen in circulating blood However mast cells do emigrate through the peritoneum into the peritoneal fluid, where they form 1 to 3 per cent of the total number of cells in normal rats [731] The numerical relations of mast cells to the other cells in peritoneal fluid are difficult to determine because of the presence of a significant number of mast leukocytes which Higgins (1953) found to have a value of 3 per cent in 1 rat [76] However Padawer and Gordon[76] found an average of 3 21 per cent in a group of 25 rats and they state that values taken from other works range from 1 6 to 7 4 per cent mast cells in the peritoneal fluid of normal rats Michels[693] shows that the mouse has practically no blood basophils, yet there are many mast cells found in the peritoneal fluid " These cellular relations are reversed in the rabbit [693 970] Code and associates (1954) report that oral administration of cortisone causes a decrease in blood basophils, whereas these cells have been known for years to disappear "during acute inflammation or stress [970]

Adrenalectomy is thought to cause the depletion of both mast cells and mast leukocytes in peritoneal fluid, but it is not known how this is effected [970] Differential resistance of these cells to adrenal hormones is further involved by certain investigators who report their inability to note any effect of cortisone acetate on mast leukocytes others report that they observe disintegration of both mast cells and mast leukocytes following the administration of cortisone [9 0]

4

Physiological Distribution of Mast Cells

The physiological implications relative to distribution of mast cells are important because mast cells are considered to be a form of storage of components for, or a readily available supply of histamine, heparin hyaluronic acid (HA),[19] [4] 5 hydroxytryptamine (serotonin),[63 576 881 945] other polysaccharides[18 1 7 8 530] and certain enzymes[61 45 8 5 1107 1108]

OCCURRENCE IN STRUCTURES

The distribution of mast cells is in general more closely related to local than to systemic functions in regard to utilization of the contents of the mast cell granules This appears to be particularly true of dermal connective tissue mast cells which Asboe Hansen[18] believes are relatively few in human skin and are more numerous in children than in adults[693] but they are very important in the formation of HA in the connective tissue ground substance[4] However heparin and other acid mucopolysaccharides (AMP) may pass freely into the systemic blood stream while relatively small amounts of other mast cell substance such as histamine and possibly, serotonin may normally be permitted to escape enzymic degradation and enter the systemic circulation A relatively large amount of the products from mast cells in the portal structures passes into the hepatic portal vein for use in the liver while a much smaller amount is used locally This idea may not be entirely correct for the stomach and intestine because these organs appear to have a comparatively sparse mast cell population when compared with the thickness and activity of the walls However it should hold for the intestinal mesenteries where vascular relations of the hepatic portal vein greatly outweigh the anticipated local demand of the meager mesenteric tissues The splenocolic mesentery which attaches the spleen to the transverse colon is well provided with mast cells that appear to be more closely related to the spleen than to the colon in the hamster[53]

Mast cells have a wide distribution in loose connective tissues[393 99 693 31 8] and are present in the connective tissue of practically all internal organs[731] Mast cells also occur in nerve sheaths[693 8 7] and in other connective tissue areas (fig 2) in the nervous system[693] Here they may also

function in AMP metabolism which occurs in myelin sheaths and in ground substance of the central nervous system[4 7] However, mast cells are absent from the parenchyma of the central nervous system in all species[855] Unfortunately these cells are ordinarily overlooked in routine preparations because the granules are soluble in most aqueous fixing solutions and are indifferently stained by routine methods such as hematoxylin and eosin

Several investigators have shown that the abundance of mast cells in a structure is dependent on the amount of connective tissue present[693] To this statement may be added the observation that there is also a vascular requirement because mast cells do not become numerous in the fat of the hibernating gland in the hamster until hyperemia appears and then in the region where conversion of white to brown fat has set in[53] It is well established that mast cells have a marked tendency to align themselves along or to 'cuff' or 'skirt' (Heller's Mastzellenhetten)[693] arterioles and capillaries[18 693 85 856 857]

Several regions of connective tissue contain considerable numbers of mast cells For example, the mesentery of most normal animals contains great numbers of mast cells and in certain instances these cells are surprisingly numerous (fig 9) The relation of mast cells in the mesentery and synovial membrane may be considered from the standpoint of specialized aspects of AMP metabolism but the AMP relations of scattered mast cells in tissues such as the skin and pancreas cannot be accurately evaluated Excellent reviews of the distribution of mast cells in different species have been given by Michels[693] for vertebrates in general and by James and McDonald[4 9] for the structures in man The functions of mast cells which occur in varying numbers throughout most of the loose connective tissues where they are accompanied by fibroblasts are extremely difficult to evaluate because mucopolysaccharides (MPS) also occur in the intercellular substance of the tissues as a product of fibroblasts as well as in the cytoplasmic granules of the mast cells which represent a very small percentage of that in the tissues

Head and Neck

Most of the mast cells that occur in this part of the body are associated with the skin or mucosa but they were found also in varying numbers in the neurohypophysis thyroid parathyroid lacrimal and salivary glands of hamsters[53] whose cornea contains numerous mast cells and stains strongly metachromatically[181] In the dense connective tissue that underlies the epithelium of the upper eyelid particularly in the proximal areas we[535] found great numbers of mast cells There are more mast cells in the dense fibrous tissue of the eyelid than in the subepithelium of the body

skin Mast cells were not concentrated or disintegrated in the areas of the Meibomian glands, but were increasingly numerous near the glands of Krause We also found considerable numbers of mast cells in the loose *fibrous connective tissue in other areas outside the globe of the hamster's eye*

The principle of the relations of mast cells and fibroblasts to mucosal and intermittent glandular secretion are discussed at some length in the sections on glandular secretion Nevertheless, we feel that in order to round out the perspective of the distribution of mast cells, the head and neck should be considered as a delimited part of the body

ORONASAL REGION

Very little of the literature on the limited amount of comprehensive, comparative work on the distribution of mast cells in the oral and nasal regions is correlated with specific regions or structural relations Indeed, we have not been able to find a single comprehensively systematic work on the distribution of mast cells in the oronasal region, or in any delimited area of the head and neck

Several investigators report that mast cells are "always remarkably numerous in the tongue [693] These cells are especially numerous in rats' tongues [49 7 1] and surprisingly numerous in the tongues of hamsters,[181] but we have found them to be no more numerous in the tongues of our hamsters than in the skin However if we had examined the submucosal structures of the attachment at the base of the tongue we probably would have found mast cells more abundant here than in the body and along the sides of this organ Mast cells are scattered throughout the dorsal sub mucosa and are numerous in the intermuscular connective tissue in the body of the tongue of the rat [7 1]

Mast cells in the mouth and nasal region follow the same general rule of distribution as they do in connection with serous and mucous glands located elsewhere in the body [5 1 8] Mast cells are associated with the secretion of mucin by glandular structures but not by unicellular glands, especially the goblet cells, which obtain the components for mucinogen directly *from their basement membranes and/or the fibroblasts underlying the basement membranes* This difference in the source of mucinogen is discussed in more detail in the section on glandular secretion The salivary glands of the mouth and the mucous and serous glands of the nasal cavity, are small, fairly simple tubular glands in the lamina propria mucosa and/or submucosa They empty their highly aqueous secretions onto the surface of the mucous epithelium

The distribution of mast cells in human nasal polyps appeared to follow the general principle because the number was more closely related to the

type of the included glands than to the growth or cystic condition of the polyp [35] Preliminary work with hamsters suggests a correlation between the number of mast cells and the secretory nature of these mucosal glands, which is similar to that of the salivary [1] [7] and Brunner s [8] glands in hamsters That is, there are a few mast cells in the tunica propria and submucosa around the tubuloacinous serous glands, more around the sero mucinous glands and a notably increased number around the mucous glands of the tongue, buccal and nasal submucosa

ESOPHAGUS AND TRACHEA

Mast cells are present in varying numbers in the adventitia of the cervical esophagus and trachea and are probably more numerous in the thoracic esophagus and trachea because of the serous external covering

The esophagus of Wistar rats is well supplied with mast cells and yielded 4 5 μg/g of histamine [7] [1] Drennan [49] also reports that mast cells are plentiful in the esophagus of rats They are reported as being frequent in the lamina propria of the Syrian hamster s esophagus [181] but the trachea probably has a greater number of mast cells than the esophagus Another group of investigators[7] [1] found that the mast cell population in the esophagus is less dense than that in the skin or tongue, and that the histamine content of these three structures is related to the density of the mast cell population in the rat

THYROID AND PARATHYROID GLANDS

Mast cells are scattered in the loose connective tissue throughout and adjacent to the thyroid (fig 11) and parathyroid glands in the Syrian hamster and are relatively increased in hypertrophy of these glands following x irradiation [1] Compton[181] observed four or five mast cells in every cross section of parathyroid glands in Syrian hamsters

SALIVARY GLANDS

Mast cells are common in the interlobular connective tissue (fig 8) and in that surrounding the salivary glands in the Syrian hamster However mast cells are decidedly more numerous in the sublingual which is characterized by most of the alveoli s being composed of mucous cells (fig 7) than in either the parotid or submaxillary salivary glands in which all the alveoli are characteristically of the serous type [5] [7]

Thoracic Cavity

The distribution of mast cells in the thoracic cavity would be expected to follow the same general pattern which has been found to obtain in the abdominal cavity with respect to serous membranes, especially because

Michels[693] states that serous membranes are rich in mast cells, that mast cells are present in the pleural fluid of mammals and that several investigators have reported the presence of large numbers of mast cells in the celomic fluid of lower vertebrates West[108] states that mast cells are numerous under the visceral pleura of beef lungs Since the work of Holmgren and Wilander in 1937, beef liver capsule has been regarded as having the highest mast cell population and corresponding heparin and histamine content of any structure However, more recent studies show that the pleura of cattle is richer in mast cells and gives the highest histamine yield of any structure (200 to 280 μg/g) [8 0] Since the pleura and pericardium are serous membranes, one would expect to find a fairly large number of well distributed mast cells to supply the anticoagulant, heparin, the vasodilator, histamine, and AMP in the form of HA, mucin and probably other substances to maintain an adequate supply of serous exudate and efficient lubrication for the thoracic viscera In the hedgehog, mast cells occur in the pericardium but are sparse or absent in the heart [404] Because of the anatomical physiological and other difficulties which would be encountered in studying serous fluid from the thoracic cavity and the comparative ease in obtaining it from the abdominal cavity most of the investigators of mast and other cells in serous fluid have used peritoneal fluid

The occurrence of numbers of mast cells in the brown fat at the base of the heart along the aorta and at the anterior third of the thymus as well as in connective tissue of the interlobar area in the thymus itself has been observed in Syrian hamsters [3]

The lungs contain numbers of mast cells [00 693] which are exceptionally large and numerous just under the pleura in beef lungs [108] The lungs of the hedgehog are fairly rich in mast cells which occurred in both the parenchyma and visceral pleura and were concentrated around the bronchial branches and small blood vessels The number of mast cells in this organ was increased by artificial hibernation but was somewhat reduced in animals awakened from hibernation [404] Hamsters are reported to have a lack of mast cells in the lungs [181] Since heparin is extracted in great quantities from the lungs of slaughter animals particularly cattle [108] sheep and hogs [1089] and a high histamine content has been found in the lungs of normal cattle and horses and in somewhat less quantity in the lungs of the guinea pig cat and dog[301] and in the lungs of asthmatics [500 904 1048] a proportionally large number of ma t cells would be expected to be pre ent especially in the pulmonary connective tissue even if the mast cells were not the source of all of the histamine and/or heparin required by the lungs

Abdominal Cavity

The peritoneum which morphologically is a simple squamous cell me o thelium lines the abdominal cavity covers all the abdominal organs and

supplies a lubricant, the peritoneal fluid for all the visceral and contiguous parietal surfaces The lubricating principles in the peritoneal fluid are essentially mucus and HA, components of which are chiefly supplied by mast cells and/or fibroblasts It is not surprising, then, to find mast cells occurring in great numbers in the mesenteric, hepatic (Glisson's) capsule and retroperitoneally, as well as within certain of the organs The peritoneal fluid of most animals is rich in mast cells which are generally presumed to have migrated through the serous mesothelial layer Here, as is usual elsewhere histamine, heparin and, possibly serotonin (5 hydroxytryptamine) are active in controlling the amount and content of the peritoneal fluid by controlling capillary dilation and inhibiting coagulation

STOMACH

The conclusions of a number of investigators indicate that 'mast cells are present throughout the digestive tract including the cecum and esophagus [693] and there is commonly a rather dense mast cell population within and under the entire peritoneum Mast cells occur in appreciable numbers in the gastric mucosa of mammals including cats, dogs and human being [7 1] but are reported to be sparse in Syrian hamsters [181] However it has been shown that there is not such a close correlation between the mast cell population and the histamine content of gastric mucosa as there is in other structures, especially the tongue skin of the body ears and dorsum of the feet [7 1 8 4 85 108] Nevertheless histamine that is released from gastric mucosal mast cells is probably highly potent Code[1] points out that since histaminase has not been found in gastric mucosa minute amounts of histamine would be much more effective than in other tissues in which this important histamine destroying enzyme occurs

Histamine and heparin play an important part in the control of the dilation of arterioles and capillaries Consequently these substances play a very important part in control of gastric secretion Code[173] shows that there is a definite correlation between the *acid secreting* regions of the corpus and fundus of the stomach and the mucosal histamine content in the cat dog and man

Gastric juice that results from histamine stimulation is highly acid and contains very little pepsin or other organic matter as compared with the gastric juice that results from stimulation mediated by the vagus nerve [134] Some investigators believe that the cells which are stimulated by histamine to produce the acid are the mucoid cells of the neck of gastric glands parietal or surface epithelial cells Babkin (1930 1950) believes that histamine has little effect on secretion by chief and mucous cells but stimulates the acid secreting cells in the gastric glands and is the strongest of all stimulants of parietal cells in normal individual [154] He also states that the histamine content is about the same in histamine gastric juice as in vagal

juice, and that histamine mediates the action of acetylcholine on the parietal cells

Code[17] cautions that any assay of histamine in gastric juice, made without a determination of the potassium content, is very likely to be inaccurate In any event, it must be admitted that in the light of certain investigations[175, 721] the part played by mast cell histamine in hepatic secretion is very difficult to evaluate

A considerable amount of significant work shows conclusively that histamine plays an important part in stimulating and thus controlling, gastric secretion but the view of the mast cell histamine relations is very much out of focus There is considerable evidence that only a minor part of the gastric histamine is supplied by mast cells, at least in certain animals Smith (1953) found that only some of the histamine in the mucosa of the stomach of cats was released by treatment with 48/80 but that this substance liberated the maximum amount of the histamine in the skin[721] Other investigators obtained similar results Wistar white rats, 24 hours after intraperitoneal injection with compound 48/80 showed surprisingly little loss in certain gastric histamine although all the mast cells were destroyed by the histamine liberator The respective histamine values obtained were mucosa of the gastric cardia 3 0 μg/g in normal and 1 0 μg/g in treated rats mucosa of the gastric fundus 15 1 μg/g in normal and 14 8 μg/g *in treated rats This is indeed surprising when contrasted with the results* obtained from the skin of the dorsum of the feet in normal rats, 6 1 μg/g, and 8 6 μg/g of histamine in the rats treated with compound 48/80[721]

INTESTINE

Michels[693] observed mast cells in the intestine of well fed salamanders (Amblystoma), horned toads and turtles but they "were extremely sparse or entirely lacking in those species which were killed during or after hibernation Several investigators report observing the tunica propria of the intestine of turtles packed with mast cells Hardy and Westbrook (1895) report that in some instances they observed whole villi studded closely with mast (basophile) cells which were so closely packed at the base of the villi as to form a basophile layer[693] The intestine of hedgehogs has relatively more mast cells than any other structure except possibly the lungs[404] Compton[181] found mast cells rare in the tunica propria but common in the submucosa around the large blood vessels in the intestine of hamsters

Mast cells were observed in the intestine and in pieces of a number of organs from a very active female painted turtle which had been kept in a laboratory for some time without food[53] Although these tissues were fixed in sublimate alcohol and stained with toluidine blue by our usual

method for hamster tissues, most of the mast cells in each structure were surrounded by a metachromatic halo resembling the "secretory halos" or "Hofen," commonly attributed to the solution of the mast granules by aqueous solutions as described by Michels[693] and others Since we have observed halos around some mast cells stained with toluidine blue and around a few stained by the PAS method in hamsters but never anything beginning to approach the percentage observed in this fasting turtle, we finally concluded that under these conditions the halo is to be expected in the course of a slow process of normal liberation of mast cell substances subsequent to intracellular dissolution of a limited number of mast granules

LIVER

The liver, when considered phylogenetically or individually as to its content of any one substance usually presents multiple, overlapping problems Consequently it should be no surprise to find variable and even contradictory statements in the literature concerning mast cell population and heparin and histamine content of the liver in the same or different species An example of a fertile basis for variable results is that sometimes it is impossible to determine from the investigator s published work whether he 'skinned the liver tissue before using it or included an undetermined amount of Glisson s capsule especially from the hilus falciform ligament and/or other tissue of mesenteric origin This is a serious point for the hepatic (Glisson s) capsule is one of the richest tissues in mast cells[414, 500, 693, 851, 108, 1094]

The findings of some of the earlier investigators, who used livers of various mammals, contribute to our tenet that some of the heparin in the liver may have had its origin in mesenteric mast cells Wilander[108] is said to have proved a relationship of heparin to mast cells by obtaining 10 times as much heparin from Glisson s capsule as from the liver tissue in cattle and by failing to obtain heparin from the mast cell free liver of rats Likewise neither mast cells nor heparin was found in the pig's aorta, but the subcutaneous tissue of the rat contained as much heparin (63 mg/k) as did the liver of cattle (56 mg/k)[500] It was also found that the sulfur content of Glisson s capsule in the horse (0 029 per cent) and pig (0 015 per cent) was much higher than in the other mammals studied Nevertheless the sulphur content in ester sulphates was found to be directly related to the mast cell content in the tissues of all animals tested[500] The parenchyma of a dog s liver is rich in mast cells, whereas it is poor in these cells in the rabbit[693]

Nakajima (1928) believes that the dog s liver (presumably the parenchyma) is rich in mast cells, whereas other investigators report that the

sparseness of mast cells is correlated with the fact that the liver has little connective tissue[693] The number of *mast cells in the hepatic parenchyma* and capsule is very small in normal hedgehogs but it is not appreciably altered by hibernation[404] The liver of young adult Wistar rats has a fair mast cell population and a histamine content of 0 5 μg/g in normal animals, but 24 hours after intraperitoneal injection of compound 48/80 the livers still *contained* 0 4 μg/g *of extractable histamine*[7 1] *Since compound* 48/80 appears to be specific for mast cells [41 79 1045] and since all the mast cells had been destroyed it appears that they supplied only 20 per cent (0 1 μg/g) of the total amount of histamine in these rat livers

The sparsity of mast cells in the parenchyma of the liver in most mammals is usually correlated with the absence in this organ of appreciable areas of loose connective tissue[693] However this correlation is not valid when it is applied to the hepatic capsule or to the interalveolar areas in the salivary glands especially the sublingual glands of hamsters[7] which are both rich in mast cells and poor in loose connective tissue whereas it appears that the liver in hamsters has a sufficient amount of loose connective tissue in the periportal and interlobular spaces[5 0 5 4] to support a rather rich population of mast cells in these areas It would be a plausible explanation of the cause of the paucity of mast cells in the liver, to assume that mast cells in the portal drainage particularly those in the mesenteries and in Glisson s capsule amply supply the liver with mast cell products

MESENTERIES

The mesenteries of mammals man and lower vertebrates are characteristically rich in mast cells[691] The Syrian hamster also has many mast *cells in the mesenteries*[181 535] *This abundance of mast cells suggests that* the mesenteries as well as the subserosa in general is a most important situation for the development of mast cells and vascular relations for transportation of the products synthesized stored and released by these cells This relation is closely related to the architectural characteristics of the mesenteries which are essentially specialized folds of the peritoneum The abundance of mast cells in the mesentery (fig 9) of most mammals may indicate that this structure is a site for the synthesis and storage of AMP Additional significance however is attached to the mesentery because of its relation to the hepatic portal system and the liver Several of the functions ascribed to the liver may depend upon the release of various stages of depolymerization of the AMP from mast cells passing into the portal vein and their subsequent concentration in the liver

Structural relations Structurally the mesentery is formed by an outer layer of peritoneum on each side which is a highly permeable, simple squamous membrane composed of mesothelial cells Between these two

serous membranes is a layer of loose connective tissue composed of collagenous elastic and reticular fibers and cells embedded in connective tissue ground substance and tissue fluid [99] through which blood vessels and lymphatics course and the deposition of varying amounts of fat occurs Fawcett has shown, by using the May Gruenwald Giemsa staining method that there are nearly as many fibroblastic as mesothelial cell nuclei in spreads of rat mesentery [7] The three kinds of connective tissue cells arise through fibroblasts from undifferentiated mesenchymal cells which also give rise to mast cells Michels[693] states that the maximal numbers of mast cells occur in the serous membranes, especially in the mesentery, where they may be found in aggregates aligned along blood vessels[8] or at the surface of milk spots in the mesentery and peritoneum [693] The milk spots are aggregates of macrophages [39] and/or monocytes distributed in the peritoneum forming the omenta and in that covering the diaphragm [7]

Serosal mast cells It has been conclusively established that mast cells store AMP and histamine and that these cells can release their stored substances very suddenly These two capacities would enable the mast cell to perform two generalized functions (1) to store certain substances which may be free in the intercellular fluid and ground substance and (2) to discharge a sufficiently great amount of its stored substance at one time to inhibit dialysis into the peritoneal fluid and at the same time greatly facilitate capillary absorption of its released substance, and, probably certain intercellular substances not of mast cell origin, as well

The liberation of the content of mast cells in the mesentery should be considered from two points of view

1 The granules contribute components of AMP for fluid secreted by the serous membranes into the body cavity which is a relation similar to that occurring in the synovial membranes (fig 12) They also increase capillary exudation

2 Mast cells in the mesentery are intimately associated with hepatic portal capillaries which conduct the products of the mast cells into the liver The mast cells are arranged around arterioles and capillaries—the same distribution that occurs in most tissues Jorpes [00] states that 'thus mast cells because of their position around the capillaries and the small blood vessels without a muscular coat have the possibility of emptying the content of their granules directly into the blood stream or into the perivascular tissue juices

Nagayo[731] reports that the serous membranes of dogs "were usually rich in mast cells and that 1 to 3 per cent of all the cells in the peritoneal fluid of normal mice and rats were mast cells He also observed that the number of mast cells was always increased by acute inflammatory stimuli and that when rats on the regular diet were fed cow s milk the mast cell

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The mesenteries of mammals, man and lower vertebrates are characteristically rich in mast cells[693]. The Syrian hamster also has many mast cells in the mesenteries[181, 3]. This abundance of mast cells suggests that the mesenteries, as well as the subserosa in general, is a most important situation for the development of mast cells and vascular relations for transportation of the products synthesized, stored and released by these cells. This relation is closely related to the architectural characteristics of the mesenteries which are essentially specialized folds of the peritoneum. The abundance of mast cells in the mesentery (fig. 9) of most mammals may indicate that this structure is a site for the synthesis and storage of AMP. Additional significance, however, is attached to the mesentery because of its relation to the hepatic portal system and the liver. Several of the functions ascribed to the liver may depend upon the release of various stages of depolymerization of the AMP from mast cells passing into the portal vein and their subsequent concentration in the liver.

Structural relations. Structurally, the mesentery is formed by an outer layer of peritoneum on each side which is a highly permeable simple squamous membrane composed of mesothelial cells. Between these two

stages of depolymerization, passing from mast cells into the portal vein and subsequently being utilized by the liver

MPS synthesized by mast cells in the mesentery of most mammals may frequently be altered to form heparin, which is collected by the numerous portal tributaries in the mesentery and conveyed by the hepatic portal vein to the liver This would account for the fact that the liver of some mammals contains a great amount of heparin[500] although mast cells are rare within the parenchyma Furthermore, detoxication of various substances by conjugation with sulfuric acid or glucuronic acid in the liver[407] may depend upon the release of these acids by the depolymerization of heparin or MPS from mesenteric mast cells in the hepatic portal system

Since the peritoneum, which lines the abdominal cavity and forms the outer covering of the abdominal viscera including the mesenteries is a highly permeable membrane the AMP and other substances which may be produced in a steady though rather limited quantity by fibroblasts in the mesenteries pass readily into the peritoneal fluid and, therefore, do not reach the liver in appreciable quantities Since the fibroblasts produce their products slowly but continuously the demands of the peritoneal fluid which constantly bathes the surface of both the parietal and visceral peritoneum, would be expected to take precedence over the demands of the liver which could receive these substances only after they had been picked up by capillaries and transported through the hepatic portal vein to the liver

The rat probably indicates more strongly than most laboratory mammals the functional relationship between mast cells in the mesentery and the liver for rats are said to have neither mast cells nor heparin in the liver[500]

The point we wish to make in this section is that mast cells in the mesenteries and possibly those in Glisson s capsule are capable of supplying substances for use in the liver for detoxication and for systemic distribution such as heparin and histamine as well as supplying part of the lubricating substances particularly HA and mucinogen to the peritoneal fluid whereas those mast cells in the parietal peritoneum contribute chiefly, if not solely to the peritoneal exudate

Mast cells in the mesentery have additional significance in the metabolism of the liver in view of the antimitotic property of heparin, which these cells supply Paff and co workers[769] believe that heparin inhibits mitosis by interfering with nucleic acid metabolism Roth (1952) suggests that heparin inhibits ribonuclease[769] Others maintain that the failure of cells other than mast cells to grow in cultures of mast cell tumors may be due to the production of heparin or a precursor of heparin[769]

The antimitotic effect of heparin in the liver could be a controlling factor in nucleic acid production and consequently, in protein metabolism of this

count increased to 7 6 per cent of all cells in the peritoneal fluid He esti mated that 3 5 per cent of these mast cells had migrated from the tissues, especially from the intestinal mucosa

From the standpoint that mast cells provide AMPs or their components for several functions of the liver, the foremost consideration is the rela tion of mast cells in the mesentery as a source of heparin and histamine As far as we know, no one has ever determined the heparin or histamine level in blood from the hepatic portal vein and, consequently, it is not known whether or not either level is constant However, it is known that both tissues[500, 603, 731] and circulating blood[0, 1084] of normal animals con tain an appreciable amount of free heparin Bassiouni[50] gives the apparent heparin content of normal human blood as ranging from 0 25 to 2 3 mg per 100 ml of plasma Species differences have been reported

The part played by mast cells in the me entery is especially significant in view of their great numbers in a given unit of mesentery and in con sideration of the relative extent and total amount of mesentery as compared with that of Glis on s capsule of the liver and the number of mast cells in the liver of most mammals

Functional relations The abundance of mast cells in the mesenteries is related chiefly to the demands of two conditions

1 Lubrication The free movement of parts of the viscera upon each other and against the parietal peritoneum creates a demand for a relatively great amount of lubricating fluid throughout the abdominal cavity This need is met by polymerized HA and mucin which are commonly supplied by mast cells[0, 1] and/or fibroblasts, and exudate which is supplied chiefly by capillaries and mesothelial cells Thus mast cells and fibroblasts ap parently supply most of the lubricating substances in the peritoneal fluid

2 Detoxication This abundance of mesenteric mast cells may al o play an important part in the detoxication of certain substances within the blood as it passes to the liver, such as sex hormones, or substances absorbed from the intestine, *e g* histamine produced within the intestine by bac teria[330] A significant and related part played by the mast cells in the mesenteries in relation to the activities of the liver is that they may provide two of the three components glucuronic and sulfuric acids which are known to conjugate with numerous substances in the liver including cer tain hormones to render them inactive

The great number of mast cells in the mesenteries of most vertebrates indicates that this structure functions as a site *for the synthesis and stor age* of AMPs as well as supplying certain components for peritoneal fluid Additional significance is attached to the mesenteries because of their re lations to the hepatic portal system and the liver Thus several functions usually ascribed to the liver may depend upon AMPs perhaps in various

has one to three mast cells associated with it These mast cells were not within the parenchyma of the islets, but were situated adjacent to the islet s capsule and near afferent blood vessels where they apparently function as a trigger mechanism for activating the islet (fig 10) This is further discussed under "Glandular Secretion

Skin

The skin has large numbers of mast cells throughout the dermis in the superficial and deep layers of the connective tissue occasionally in the epidermis [693] inside and outside the hair follicles [49 693] and around the sebaceous and sudoriferous glands Mast cells are much more numerous in and under the loose, relatively thin skin of the dorsum of the feet and the ears than on the back of rats [721]

Dermal mast cells have been shown to release histamine [6 721 797 8 108] heparin [18 854 1095] HA or its precursors, [1 150] and other important compounds to form the HA containing mucinous connective tissue ground substance [4]

Collagen occurs in the skin in considerable quantities Whether or not an appreciable amount of it is derived from mast cells in the skin is an unsettled question However it has been found that the repeated administration of estrogens to virgin female hamsters was followed by increased mast cells AMP and collagen in the mammary gland [530] Mast cells in the skin could well be the source of hyaluronic and chondroitin sulfuric acids [91] for collagenous connective tissue or for chrondroitin sulfate B which is considered the probable cause of metachromasia of the hair papilla and external follicular cells[707] and as a source of constituents of ester sulfate in the formation of keratin [1000]

The significance of the skin as a source of mast cell products becomes apparent when one realizes that the skin of a normal mouse represents at least 16 per cent of the animal s total weight and contains about 40 μg/g of histamine,[797] whereas in the normal rat the skin from the belly has an equally high histamine content (40 μg/g) ,[797] skin from the dorsum of the body 27 9 μg/g and skin from the dorsum of the feet 61 μg/g [7 1] Abdominal skin from the guinea pig contained only 3 μg/g of histamine [97] The above values for the rat and the results of other investigators work show that mast cells and histamine are not equally concentrated in all parts of the skin of the same animal [7 1 797] It is also claimed that the only skins that have a high mast cell number are those of the cat mouse and rat [797] However, Harris (1927) reported finding histamine concentration that reached 28 μg/g in normal human skin [867]

Since Perry[797] found that compound 48/80 which is generally conceded to be almost specific for mast cells [7 1 797 83 869 896] liberated 90 per cent

organ Paff and co workers[769] support this idea by concluding that the anti mitotic reaction of heparin, which they point out has been known for years functions in this respect by interfering with the metabolism of nucleoproteins The antimitotic property of heparin has special significance in relation to protein metabolism of the liver cells because the hepatic cell are similar to the columnar cells of villi in the small intestine in being subject to variations in the amounts of ribonucleic acid (RNA) and other proteins *in the cytoplasm Increases in RNA and proteins in the cytoplasm of ex* posed intestinal columnar cells do not cause mitosis because these cells do not divide It is possible that the high content of heparin in the liver of most animals acts as a controlling factor in mitosis and enables the hepatic cell to store considerable amounts of RNA without stimulating mitosi

Glisson s capsule Whether mast cell substances from the heavily populated Glisson s capsule pass into the portal system or into veins in the parieties and/or diaphragm has not been satisfactorily established Michel s[694] review of the results of various investigators attempts to demonstrate the source of the arterial blood supply of Glisson's capsule shows that it is highly variable However very little attention appears to have been paid to the venous drainage of this morphologically complicated structure Incidentally we have never observed a significantly great number of mast cells in the delicate capsule in studying cross sections of hepatic lobes of the hamster

The embryological development of Glisson s capsule and its covering of peritoneum from the mesenchyme of the primitive septum transversum (later ventral mesentery) [694] suggests the probability that its venous drainage is by portal tributaries and/or parietal vessels The fact that blood from the gall bladder drains into the portal system supports the idea that part if not most of Glisson s capsule has venous connections with the portal system

PANCREAS AND ISLETS

The distribution of mast cells in the pancreas follows the general plan for compound glands such as the salivary and mammary glands in which these cells are situated periductally in the loose interalveolar and para alveolar connective tissue[181, 5 1, 30, 535] However the pancreas has fewer mast cells than the salivary glands[5 1 5 7, 30, 53]

The islets of Langerhans have received very little attention in connection with mast cell relationships Compton[181] states that no mast cells are seen in or near islets in the pancreas of hamsters However using an entirely different method of fixing the tissues and staining tissue mat impregnated non serial sections cut at 6 μ we[533, 535] concluded that in most instances, in our inbred colony of normal hamsters of both sexes each islet

fense against it, for the number of mast cells did not increase in tar cancer in rabbits

These rather simple experiments indicate that histamine was released with the first application of tar to mice and caused vascular dilation followed by edema and an increase in mast cells The dermal cells as shown by Foerster (1924 and 1925) who used trypan blue as a vital stain were not changed appreciably until after a few applications of tar when the number of dye laden dermal cells was increased[1111]

FETUS AND NEWBORN

West[108] states that tissue mast cells do not appear in the fetus until a short time before birth and until the mast cells appear in the fetus histamine is almost entirely absent Audry (1896) reported that he found mast cells well established within and about the hair follicles in the early newborn[693] Heller (1904) states that these cells persisted even after loss of the hair through pathological changes[693] As would be expected Huguenin states that mast cells appear in the skin much earlier than in the mammary glands[693]

Compton[181] was unable to identify any mast cells in spreads of connective tissue from 10 1 day old hamsters However he found a few mast cells in 3 to 4 day old hamsters Apparently the skin of growing individuals requires more mast cell products than that of adults because Brach (1925) for man and Bates (1935), for rats showed that the skin of young individuals contained a much higher proportion of mast cells than that of adults[693]

LYMPHOID TISSUES

Lymphoid structures with the possible exception of the vermiform appendix of man and the spleen of man and various animals in which there is a great amount of other tissue are characterized by being preponderantly composed of lymphocytes supported by reticulum Lymphoid tissue is more readily studied if it is grouped on an architectural basis as in the following manner (1) lymph nodes which include the so called lymphatic glands tonsils and human vermiform appendix (2) intestinal lymphoid tissue including solitary nodules Peyer s patches various patches of lymphoid tissue in the cecum and aggregates of lymphocytes and plasma cytes within the lamina propria especially in the intestinal villi (core) and as special addenda the sacculus rotundus tonsilla ileocecalis major and minor and the vermiform appendix of the order Lagomorpha (rabbits hares and pikas) (3) thymus and (4) intervascular lymphoid structures which include the spleen hemal nodes and hemolymph nodes

of the histamine that is contained within the skin of rats, it is apparent that the mast cell population was very high in the skin of the rats used Several investigators have shown that there is a *high correlation between* the histamine content and the mast cell population in the skin of various mammals, particularly the rat[71, 797, 8, 869, 896]

Carcinogenic processes during the formation of skin cancers are commonly attended by increased numbers of mast cells as various investigators have observed following the application of chemical carcinogens[910, 1111] Cramer and Simpson[16] observed a marked increase in the number of mast cells in the skin of mice following the application of methylcholanthrene Since this increase in mast cells was very pronounced long before the appearance of the carcinoma and disappeared in the tissue with the approach of the growing cancer, they suggest that this increase in mast cells was related to epidermal hyperplasia rather than to the initiation of cancer development The most pronounced accumulation of mast cells was in the deeper contiguous dermis which had not been invaded by the developing cancer whereas the mast cells had disappeared from the cancer itself Since the mast cells were greatly increased in the hyperplastic tissue disappeared in the path of the developing cancer, but remained in the contiguous dermal structures they suggest that the increased number of mast cells was associated with some sort of a defensive process against the cancer's development

In the light of the effects of estrogen on mast cell activities in the mammary glands of hamsters[530] as previously explained it would hardly appear that the mast cells were playing a defensive role in the carcinogenesis induced by methylcholanthrene Rather the mast cells would be expected to supply substances especially histamine, to induce hyperemia, *increased capillary permeability and edema which would aggravate rather* than impede carcinogenesis It has been shown that when the skin on the backs of hairless mice is caused to thicken by percutaneous or subcutaneous application of estrogen extracts of the hypertrophic skin resemble extracts of umbilical cord in viscosity[57] In all probability this viscosity in mouse skin is due to increased AMP chiefly HA produced by *mast cells and/or fibroblasts*

Woglom[1111] states that Dreifuss and Block (1922) after the first application of tar to the backs of mice observed edema and striking dilation of the blood vessels especially of the capillaries They also increased in number and were sometimes accompanied by increased numbers of mast cells Peyron (1923) observed similar vascular changes in the skin of tarred mice[1111] However he maintains that the increased numbers of mast cells played no *special role either in the genesis of cancer or in de*

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Lymphoid tissues are particularly susceptible to x rays[89, ?31, 535] and with certain exceptions (e g mouse thymus), to gamma ionizing[89] and many other forms of irradiation However, Hughes and Job (1937) found it impractical to administer a sufficiently strong dosage of x rays to produce total involution of all the scattered nodes and other lymphoid tissues without endangering the life of the animal [51]

The pharmacological relations of mast cells to lymphoid tissue appear to be limited chiefly, but not entirely, to the histamine and heparin stored within the cytoplasmic granules which Riley[8] states are the only pharmacologically active substances known to be present in the mast cells Histamine and heparin chiefly affect the lymphoid structures by inciting hyperemia and increased permeability of capillaries and ground substance Thus the products of mast cells are concerned with dilation and general tonus of the vessels and with inhibiting coagulation of lymph,[954] as well as blood within and around the lymphoid structures These relations strongly indicate that one should consider the occurrence of involutionary or degenerative conditions in lymphoid tissues and the changes in AMP metabolism which accompany them when considering the part played by mast cells in relation to the activities of lymphoid tissues

Lymph Nodes

Mast cells were not present in the capsule or in the germinal centers of lymph nodes but were aggregated within the cortex and medulla in normal rats [09] We have verified this observation in rats and have similarly observed that although mast cells are not numerous they are scattered throughout the medullary cords and sinuses in various lymph nodes in normal hamsters Mast cells are more numerous in the pancreas A elli (largest of the mesenteric nodes) the substernal thymic nodes and in some of the cervical nodes than in the axillary and inguinal nodes in normal hamsters Various investigators report finding numerous mast cells in the capsules and trabeculae occasionally free in lymph sinuses in the medullary strands of lymph nodes and in the tonsils However Meyer (1918), found them more common in hemolymph nodes than in lymph nodes [693]

Smith and Wood [954] found mast cells normally occurring in the sinuses of popliteal lymph nodes in normal rats They also observed that within 5 minutes after inoculation with a type of Pneumococcus into the plantar pad of rats, mast cells in the sinuses of the popliteal lymph node were vacuolated and almost devoid of granules however free granules were scattered throughout the sinuses and soon began to disintegrate The sinuses in which mast cells were absent or had been depleted were usually sites of fibrin formation Apparently although they did not realize it at

the time, Smith and Wood's work is one of the earliest as well as one of the few instances in which the cytolytic effects of a histamine liberating agent on mast cells in peripheral lymph nodes is recorded

Since the sinuses of these nodes in which mast cells were absent or had been depleted were usually sites of fibrin formation,[9 4] it would appear that the absence of some mast cell substance, possibly heparin is responsible for the formation of fibrin

The actual MPS which inhibits fibrin formation has not been established, but there are several forms of AMP that could have this function The most obvious anticoagulant is heparin which has been recognized as an anticoagulant of lymph[954] as well as blood[600] But, as stated above, certain other MPS are also anticoagulants Sundberg and colleagues[996] describe an anticoagulant in human umbilical cords but they do not identify the AMP as heparin We have not been able to determine if the antifibrin AMP from mast cells in the medullary sinuses of lymph nodes is heparin, HA or another form of AMP However, the presence of mast cells in the medullary cords of lymph nodes adds support to the significance of the tenet that the granules of mast cells are composed chiefly of an essential form of AMP

Intestinal Lymphoid Tissue

Intestinal lymphoid tissue lacks afferent lymphatics It is characteristically situated in the lamina propria but usually bulges into the muscularis and well into the mucosa of the intestine Certain of these structures, including the vermiform appendix are highly specialized in lagomorphs The luminal side of the lymphoid patch is covered by a lymphoepithelium in which the epithelial cells, where present are atypical They have been foreshortened and characteristically lack the striated border and a complete basement membrane They have intercellular perforations which, in the rabbit appendix connect with the sublymphoepithelial sinuses [0] and these epithelial cells are in the process of being crowded out by lymphoid cells and phagocytes In fact, the lymphoepithelium covering intestinal lymphoid tissue is modified so that it serves as a sort of specialized gateway through the intestinal wall This permits the escape of substances (probably including bacteria and viruses) which cannot be absorbed through normal columnar epithelium due to the protective covering of mucus held by the striated border

Thymus

Several writers including Maximow (1909), Schaffer (1910) and others state that the thymus has a conspicuously high proportion of mast cells[319] during its functional and involutionary phases[691, 798] Michels[691] states

that the factor that causes this high percentage of mast cells to be present in the thymus is not known Compton[181] found mast cells uncommon in the thymus of hamsters, except in fatty infiltration when these cells became numerous However, it is known that total body x irradiation increases the number of mast cells in the thymus[09 5 6] (fig 17) Maximow (1909) *believes that thymic mast cells are developed from immigrant lymphocytes which migrated into the developing thymus during its early histogenesis and Dantschakoff (1908) apparently believes that in birds* only the first generation of the thymic mast cells arise from lymphocytes[693] However, it is not clear whether she believed that the additional mast cells arose by mitosis from the first generation or from other sources, such as undifferentiated mesenchymal cells Certain other investigators believe that the greatest number of mast cells is present during the early involution phases of the thymus and that at this time plasmacytes and eosinophils also reach their maximal population[693] Lehner[799] (1924) and Harland[403] believe that mast cells differentiate from lymphocytes or from lymphocytic cell types in the normal thymus of the rat, but Peter s[98] work on the thymus of the hedgehog does not support this conclusion

Mast cells appear late in fetal life,[105] are very noticeable in and around the thymus in postnatal rats[403] and are more numerous in the thymus in early rather than late childhood[693] At least one investigator Weidenreich (1912), suggests that the heteroplastic differentiation of its thymocytes into mast cells accounts for the major part of the reduction in the size of the thymus during involution[693] Weil (1913) admitted that he was unable to explain the development of thymic mast cells but thought that many of them were plasma mast cells which had developed from plasma cells[693]

Peter[798] suggests that there is no actual increase in the number of thymic mast cells during involution in the hedgehog The number remains constant but appears to increase because the greatly reduced thymic tissue contracts the connective tissue and thus draws the mast cells closer together This, of course could markedly increase the number of mast cells in the field of vision It is not surprising therefore that Peter concluded that the maximal number of mast cells is related to the maximal depletion of the thymic tissue during involution

The thymus has been depleted by a relatively wide assortment and number of agents but unfortunately comparatively few of the investigators were interested in the mast cell relations to the thymic changes Many of the agents which produce thymic atrophy probably cause an increase in the number of mast cells within and around the thymus[89 09 r 6 9] as well We[5 6] found that this idea was valid for the effects of x irradiation but, although both cortisone and starvation produced extreme

depletion of the thymus, neither significantly altered the number of mast cells within it.

Depletion of the thymus may be related to age changes or to "accidental involution," which includes about all experimental possibilities. In general, those agents that interfere with protein anabolism, especially as pertains to the nucleic acids, deplete the thymus, which is the more sensitive, and, if continued, will deplete the other lymphoid tissues as well. Even in the presence of "otherwise adequate diets," pyridoxin deficiency, or even insufficiency, produced marked thymic atrophy. Deficiency of pantothenic acid, thiamin or riboflavin produced similar but milder depletion of the thymus in female rats used before 'age involution' had started.[844]

Numerous other agents deplete the thymus, e.g., x-irradiation,[89, 99, 566] fast and slow neutrons and isotopes,[89] cortisone[706] and many of the chemotherapeutic agents used in treating leukemia and cancer. Bloom[89] reports that mice which received daily total body irradiations of 1.1 to 8.82 r for periods that varied from 4 to 16 months had a striking increase in the number of mast cells in the thymus, other depleted organs and in the connective tissue surrounding the depleted structures. He also reports that 500 rep of externally applied beta rays, which is sufficient to kill 20 per cent of the animals, has no effect on the thymus of mice, but whether or not the mast cell population is altered is not disclosed.

We[566] made some observations on the number of mast cells in the thymus of 93 male and female Syrian hamsters under different conditions. The thymus of each animal was removed intact, arranged with the lobes extended, fixed on a strip of stiff paper and sectioned at 6 μ. The number of mast cells in each cross section of the thickest region of the two lobes was counted and the numbers averaged for each individual. The results for each of the eight groups are tabulated in the original article.[566] In the group of 18 normal hamsters that comprised 12 males and 6 virgin females 38 to 387 days old, 9 had an individual average of 18 to 25, 8 had 26 to 50 and 1 had 65 mast cells per cross section of a thymic lobe. The range for the group was 18 to 65. There was no significant difference in the number of mast cells in males and females. The values for 12 pregnant, 7 postpartum and 7 males 78 to 101 days old injected with triturated viable fetal tissue did not differ significantly from those of the control group. Age differences that ranged from 38 to 387 days did not appreciably affect the number of mast cells per cross section of a thymic lobe in any of the above groups of hamsters.

Two hamsters bearing both viable and 1 or 3 partly resorbed fetuses had a definitely increased number of thymic mast cells. Since 7 male

78 to 101 days old, which were injected with triturated tissues of dead fetuses did not show an increase over the control number of mast cells, we[5, 6] concluded that absorption of fetal tissues was not the cause *per se* of the increased number of thymic mast cells in the two pregnant females

Cortisone acetate (Merck, 0 15 mg/100 g in 3 to 21 subpannicular injections) reduced the size of the thymi as much as 85 per cent, but scarcely affected the number of mast cells in 9 hamsters 31 to 300 days old Inanition, effected by reducing the amount of regular food until 30 to 40 per cent of the initial weight was lost produced extreme depletion of the thymus in 5 male hamsters 92 to 207 days old However, it did not reduce the number of mast cells as determined by counts in cross sections of thymic lobes[5, 6]

One to three injections of adrenocortical extract (Upjohn, 50 dog units/100 g body weight) over periods ranging from 1 to 7 days produced a slight depletion of the thymus in 11 male and 4 virgin female hamsters, 80 to 229 days old However, it failed to increase appreciably the number of thymic mast cells[5, 6]

Spleen

The spleen by virtue of its peculiar histological architecture and being interposed by sinusoids between branches of the systemic arterial system and tributaries of the hepatic portal system is potentially a strategically located organ in the blood vascular system Consequently, it has multiple functions that are often little appreciated Nevertheless rabbits[514] other experimental animals and men appear to be able to live very happily after its successful removal

The literature is fairly extensive on hematopoietic changes induced in the spleen by various agents but relatively little attention has been directed to the mast cells This omission of observations on the mast cells should be attributed chiefly to the fact as admitted by one investigator[7, 9] that a fixation and staining technique to demonstrate mast cells properly was not used

The distribution of mast cells in the spleen follows the general pattern with regard to a visceral organ and its adnexa Several writers maintain that mast cells are numerous in the capsule trabeculae and adventitia of the arteries and may occasionally be found free in the venous sinuses[693] We have observed in hamsters a few mast cells in the capsule and within the spleen and an abundance of them in the mesenteries that attach the spleen to the pancreas stomach and colon

Bloom[89] reports that chronic irradiation produced depletion of splenic white pulp and increased erythropoiesis in the red pulp These changes appeared in the spleen of mice after daily administration of 8 8 r of x rays

for 6 months and increased progressively in numbers along with the increasingly reduced lymphocytopoiesis and increased erythropoiesis. Mast cells in the spleen of these chronically irradiated (8 8 r daily) mice had a *maximal increase in numbers reaching 4 5 times that of normal non irradiated mice*

Holmgren and Wilander (1937) studied mast cells in the spleen of cattle dogs, hogs, horses rabbits and rats[93] However these cells occur only in the capsule and are absent in the parenchyma of the spleen in the hedgehog[404]

REPRODUCTIVE SYSTEM

Comparatively little work has been done on the occurrence of mast cells in the primary reproductive system of either sex Nevertheless several investigators have observed a dense population of mast cells near the larger blood vessels in the connective tissue of the uterus but few of these cells occur in the mucosa of normal uteri or in gravid puerpural or inflamed uteri[693] Most of the experimental work on altering the number of mast cells in reproductive structures has been restricted to the effects of estrogens progesterone and/or testosterone on the uterus and mammary glands

Since mast cells are fairly abundant in the comb and wattles of roosters[71, 1004] one would expect to find these cells present in or around the glans penis clitoris nipples and other erectile tissues in mammals man and probably lower forms This assumption is supported by the report that "mast cells are always remarkably numerous in the glans penis[693]

The prepuce of rats is rich in mast cell[49, 708] Supravital staining with neutral red colored the mast cells selectively and showed that these cells were abundant *in the preputial glands in the connective tissue surrounding* the ducts However they were not so numerous in the interacinar connective tissue and were not found in rats in the capsule of these glands[09]

GLANDULAR SECRETION

Glandular secretion is generally conceded to be controlled primarily by parasympathetic nerve fibers However Bayliss and Starling (1902) and later investigators have shown that humoral (blood borne *e g* secretin)[30] or chemical[63] control of pancreatic secretion is also involved and that these effects may be demonstrated in certain other glands[60, 30] Other *investigators believed that in addition to the well known parasympathetic* stimulation sympathetic fibers from the celiac plexus may cause a limited flow of pancreatic juice[60] Stavraky[9 6] found that intravenous injection of histamine activates a true secretion and it presses out the contents of the alveoli and ducts in the parotid and submandibular salivary glands

of the dog Just how histamine "presses out the contents of the alveoli and ducts is not explained but it probably is accomplished indirectly through hyperemia within the gland Another interesting point is that several investigators have shown that glands, in general are relatively radioresistant,[86] but that sebaceous glands are very sensitive to epilating doses of x rays, while sweat glands are nearly twice as resistant[89]

Whether or not mast cell products play a direct part in the discharge of secretions is questionable for the chief function of these cells appears to be related to the production, rather than to the discharge, of secreted substances

The view that certain substances in mast cell granules are available for the synthesis of mucinogen or mucin is not entirely new Raundnitz (1883), and later investigators including Staemler (1921) had a general grasp of this idea[693] However, the significance of Staemler s view was that mast cells are unicellular, connective tissue glands which synthesize and supply the mucin which he believed was necessary for the production of the 'Kitt substance,' or interfibrillar cement substance Harris (1910) believed that mast cells not only contain mucin but supply the connective tissue with this substance[693] He was so certain that these cells store and release mucin that he proposed the name mucinoblast to supplant the term mast cell Certain other investigators maintained that there is no relation between mast granules and mucin After a series of brilliant experiments in which mast cells and mucin were compared by various staining and solubility methods Clowes and Owen (1904) were unable to find any evidence of a single chemical relation between the content of mast granules and mucin[693] We think that the inability of these investigators to demonstrate chemical relations between mast cell granules and mucinogen or mucin should be attributed to the fact that mast cell granules contain several MPS which are nearly components and may be used in the synthesis of mucin, heparin possibly HA and probably other substances In addition mast granules have been shown to contain histamine in an inactive form

The significance of mast cells within and around secreting glands apparently lies in their ability to synthesize store and suddenly release (fig 7) the pharmacologically active substances necessary to produce hyperemia with increased capillary permeability At the same time this facilitates local pooling and utilization of these extravasated substances by the gland of the substances thus provided by both the mast cells and the blood stream Thus the relations of mast cells to glandular secretion may be considered to be the result of two primary functions of these cells (1) the release of histamine and heparin for the purpose of producing hyperemia by capillary dilation with consequent leakage of proteins, serum and other substances into the intercellular fluid for speedy absorption and

utilization by the gland, and (2) the release of MPS, by which heparin or other AMPs especially those for synthesis of mucinogen, are suddenly made available for utilization by the secreting gland

The release of heparin from mast cells and, perhaps, with the addition of other components of MPS prevention of coagulation of tissue fluid, lymph and blood thereby facilitate the dissemination of amino acids proteins, serum and other substances in the plasma which may have passed through the walls of the dilated capillaries Certain of the AMP released from mast cells are believed to be utilized directly by certain glands such as the salivary glands[5, 7] in the synthesis of mucinogen

A number of investigators believe that mast cells have some important part to play in aiding the normal secretory functions of glands but very few of the explanations given for these relations consider that mast cell production of hyperemia has a significant bearing on glandular secretion and/or other activities The tenet of Brack (1925) that normal growth of certain connective tissues is dependent upon the presence of mast cells is generally considered to be problematical[693] That these cells are well established within and around hair follicles apparently is a generally accepted fact however[693]

Mammary glands the thymus and certain mucin secreting glands show significant variation from the general rule that the mast cell population is related to the amount of loose connective tissue present[693] Certain epithelia especially the unicellular glands such as goblet cells in the columnar intestinal epithelium appear to depend almost entirely upon fibroblasts for their supply of mucinogenic components Therefore they appear to be quite independent of the mast cells as a source of components for the synthesis of mucin

The increase in number and disintegration of mast cells in the sublingual salivary glands (fig 12) and mucous areas of Brunner s glands (fig 13) and their absence in connective tissue underlying other mucin secreting epithelial cells further indicate that an important function of mast cells in mucous glands is to synthesize and store AMP Contrarily there is no apparent necessity for mast cells to be present in those areas in which the ground substance of the connective tissue contains AMP This idea is well illustrated by simple epithelia which even though very active in mucin secretion, *e g* the lining of flask shaped glands in the rabbit appendix[0] do not require a concentration of mast cells because each cell rests upon the basement membrane which can draw and retain AMP from the underlying tissue

Mast cells do not appear to play an important part in the formation of the intercellular substance which cements epithelial cells together and forms the basement membrane that connects the epithelium to the under-

lying connective tissue[393] That is because the components of this substance, apparently, are supplied chiefly by fibroblasts However, mast cell products probably contribute histamine and, indirectly, to the formation of intercellular substance and mucinogen in certain epithelia which secrete mucin rather than form keratin In some of the mucosal epithelia such as those that occur in the stomach and uterine cervix, any or all of the cells may secrete mucin[393] In other mucosae such as those in the intestine, however, certain individual (goblet) cells assume the function of storing and suddenly releasing by rupturing, the stored mucin Goblet cells may be found in all stages—from early conditions to distended, ruptured and depleted conditions Thus, it is obvious that the production and storage of mucinogen by unicellular glands such as goblet cells and by glandular areas of epithelium as found in the stomach and cervix uteri, is an essentially continuous process Although a fair representation of mast cells may be present in the connective tissue that underlies the basement membrane, they apparently play little or no part in supplying components for the formation of mucinogen excreted under these conditions which apparently is supplied almost entirely by fibroblasts

The number and relation of mast cells to mucin secretion is dependent upon the amount of ground substance available in the contiguous connective tissue Thus areas of epithelium such as the lining of the flask shaped mucous glands in the rabbit s appendix,[0] which consist of numerous goblet cells among the simple columnar cells most of which are actively secreting mucinogen depend chiefly upon fibroblast produced ground substance for their supply of components for synthesizing mucinogen For this reason mast cells are scarce in the contiguous connective tissue This observation is contradictory to the relation that exists in other mucus secreting tissues, such as the sublingual salivary glands and Brunner s gland in the hamster We find in these mucous glands a significant increase in the number and disintegration of mast cells as well as a direct relation between them and the mucin secreting alveoli The function of mast cells as synthesizing and storage cells for AMP is evident in those glands in which mucous alveolar cells are numerous and compose most of the gland and in which there is comparatively little stroma and connective tissue and, consequently very little ground substance from which to obtain AMP or its components, as in the salivary glands[5 7]

Another approach to the solution of this question involves the functional activity of the gland itself that is whether it secretes more or less continuously or chiefly at irregular intervals in response to exogenous stimulation Mast cells apparently play an important part, as indicated by the numbers present, in promoting normal functions of those glands and certain non glandular *structures which produce their products intermittently*

cyclically, periodically or experience a 'crest' of productivity However, this is only one view of the problem, for mast cells increase in number in the thymus with the onset of age or accidental involution of this structure[51,56,57,693] Michels[693] after citing the work of numerous investigators states that the causative factor responsible for the great increase in the number of mast cells in the thymus is 'unknown and unexplained' However, it appears to us that the increase in the number of mast cells in the thymus during involution suggests that these cells are synthesizing and storing AMP from substances liberated during the process of involution

Salivary Glands

Mast cells are common throughout the connective tissue of the three paired salivary glands in the Syrian hamster[57,693] Nevertheless the amount of interalveolar and especially intercellular connective tissue is surprisingly limited so much so that the mast cells are sometimes squeezed between the structures

Preliminary work indicated that in hamsters mast cells are more numerous in the sublingual salivary gland which contains only mucinous acini (fig 7) than in either the submandibular or parotid glands which typically contain only serous acini This lead suggested the desirability for further investigation to determine whether or not mast cells are related to mucinogenesis in the salivary glands

The Syrian hamsters used in the following series of experiments were all adults at the time the experiments were initiated but they were 84 to 447 days old when killed All of these animals were from our colony and had been selectively inbred but not consistently brother sister mated, for nearly 10 years

SALIVARY GLANDS OF NORMAL ANIMALS

Burstone[15] states that in normal mice the sublingual salivary glands stain more intensely than the submandibular glands but he does not relate this finding to mast cell population as is the case in the Syrian hamster[57]

Details of the technique employed to study mast cells in the three paired salivary glands of the hamster are given with the experimental results in the next section We[57] have observed that there are more mast cells in the salivary glands that contain mucin secreting alveoli (sublingual) than in those containing serous alveoli (parotid and submandibular) in normal hamsters The salivary glands of the Syrian hamster are remarkably nearly free of mixed alveoli Only 2 hamsters out of all the control and experimental groups studied had mixed glands These 2 hamsters had small

mucin secreting areas in the submandibular salivary glands which were correlated with the presence of an advanced, transplanted basal cell carcinoma (HC 1) The number of mast cells in the mucin secreting glands was actually 2 to 3 times that in the serous glands[5, 1] The interalveolar arrangement and physical condition of the mast cells in the salivary glands of hamsters also indicate that mast cells contribute MPS to mucin secreting alveolar cells In the sublingual (mucous) glands of normal hamsters 86 to 90 per cent of the mast cells were adjacent to or actually touching alveolar cells, and 10 to 14 per cent were farther away in the interstitial or interalveolar connective tissue In the submandibular and parotid (serous) glands, the relations were nearly reversed, however, with only 16 to 25 per cent of the mast cells adjacent to alveolar cells and 75 to 84 per cent in the interstitial or interalveolar connective tissue[5, 7]

The granules of mast cells adjacent to alveolar cells in the mucin secreting sublingual glands were usually free or being freed the mast granules of those cells more distant in the connective tissue were seldom free, thus indicating inactivity of these mast cells

The greater number of mast cells in the sublingual gland and their increased rate of disintegration as compared with those in the submandibular and parotid glands support the tenet that mast cells have a function in providing substance for the synthesis of mucin by the sublingual gland These observations suggest that in addition to the usual relations of mast cell products, especially histamine and heparin,[854, 103] to hyperemia there is normally a greater demand by mucin secreting alveoli for essential AMP stored in the granules of mast cells than by serous alveoli There were relatively more mast cells releasing granules in the sublingual than in the other two paired glands The fact that we consistently observe more mast cells in normal glands that contain mucin secreting alveoli than in those that contain serous alveoli strongly suggests that the distribution of mast cells is correlated with special storage components supplied by these cells for the synthesis of mucin by the alveoli since these alveolar cells do not have available quantities of MPS in the connective tissue for the simple reason that there is scarcely any connective tissue between the alveolar cells in the salivary glands

Mast cells were more numerous more irregularly shaped—had longer cytoplasmic processes and a higher degree of dispersion of freed granules in the sublingual which contained only mucous alveolar cells than in the submandibular or parotid gland which contained only serous alveoli in each of the control hamsters considered here and in those observed in connection with other experiments

The number of mast cells in the 21 control hamsters that were used in the irradiation experiments averaged at least twice as many in the sub

lingual as in the submandibular gland The e control hamsters included one or more litter mates of most of the animals that formed the various ex perimental groups The frequency distribution of the ratios showed that 6 of the 21 controls had 3 to 6 times as many mast cells in the sublingual as in the submandibular gland The mean ratio of mast cells in the subman dibular sublingual and parotid glands of the 21 control hamsters was 1 2 0 7, re pectively [5,7] The number of mast cells in the three salivary glands of 5 male and 6 female controls did not differ significantly, and sex differences were not observed in the terminal alveoli of the submandibular gland in either the control or experimental hamsters The only significant effect that might be attributed to age was noted in 2 hamsters less than 54 days old in which the sublingual gland contained fewer mast cells than the submandibular We are unable to offer an explanation for this dis crepancy in animals which had been weaned approximately 15 days

SALIVARY GLANDS OF EXPERIMENTAL ANIMALS

In the quest for information on the distribution and relations of mast cells in the mucigenous, paired salivary glands we submitted 21 hamsters to total body x irradiation 10 to low protein diet and 36 to implantation with sarcoma and carcinoma

Total body x irradiation The effects of irradiation on mast cells and glandular secretion vary considerably with the duration of the postirradia tion period as well as with the dosage The following description is based upon a group of 32 male and 22 female hamsters which were individually expo ed to a single x irradiation of 400 995 or 1200 r as determined in air with a Victoreen r meter However no allowance was made for scat ter from the metal cages Each hamster was confined with ample turning room in a no 4 hardware cloth, a rectangular cage that had dimensions of 2 5 by 3 5 by 5 in which was closed by a 2 5 by 3 5 in lid of sheet gal vanized iron The factors used were 200 kv 152 v and 20 ma with a 1 mm aluminum filter at a 36 cm target distance very much as de cribed in an earlier work [5,6] All of the animals were killed 3 to 18 days after irradiation by cervical fracture (fracture of the third cervical vertebra)

The submandibular sublingual and parotid glands of each side were aligned on a piece of stiff paper before fixation in sublimate alcohol so that when cut each section included tissue from all three salivary glands

The glands were routinely embedded in ti suemat (Fisher) sectioned at 6 μ and stained with toluidine blue O (National Aniline Division, Cert No NU 9) in water having a pH of 7 0 That the granules of mast cells of hamsters are insoluble in water after fixation in sublimate alcohol was shown by comparing the number of mast cells in mounted ections that were immer ed in water at 37 C for 7 days with con ecutive sections from

the same block of tissue which were exposed to 75 per cent alcohol, but not to water, for the same period of time

Supplementary methods used to compare granules of mast cells with mucin or mucigen in alveolar cells included the periodic acid Schiff (PAS) test for water soluble and insoluble polysaccharides[347] the Feulgen method for thymonucleic acid,[191] saffranin for metachromasia and treatment with ribonuclease[987]

To determine the location of mast cells within the sublingual gland the deparaffinized sections were placed in water before staining to remove the water soluble mucin and demonstrate the cytoplasmic reticulum in the alveolar cells which is insoluble in water and usually retains the toluidine blue This method sharply differentiated both mast and alveolar cells

The PAS positive cytoplasmic reticulum in the mucin secreting cells of the sublingual gland resembled the granules of mast cells in being insoluble in water following fixation in sublimate alcohol and in being metachromatic when stained with toluidine blue or saffranin However, the granules of this reticulum differed chemically from the mast granules because they gave a positive PAS reaction by either the hydrous or the anhydrous methods, whereas the granules of mast cells were PAS negative in all hamsters with the exception of one which bore the basal cell carcinoma (HC 1) In this hamster mast cells in all the salivary glands, pancreas, thymus and mesentery were PAS positive These observations agree with the statement of Lillie[608] that most mast cell granules do not give a positive PAS reaction Compton[181] suggests that the failure of mast cell granules of hamsters to stain consistently with the Hotchkiss PAS reagent may be due to the presence of a trisulfuric form of MPS

To compare the number of mast cells in the sublingual gland with that in the submandibular and parotid glands the number of mast cells in four fields of the microscope (× 200) was totaled for each gland Since the number of mast cells in the submaxillary gland was intermediate between that of the other two glands the ratio of the total number of mast cells in the submandibular to the total in the sublingual and to that in the parotid gland was determined for each individual To compare the number of mast cells in the salivary glands with that in other organs the liver pancreas and thymus of each hamster was sectioned and routinely stained with toluidine blue

Results of irradiation Total body exposure to x irradiation of 400 995 or 1200 r did not significantly alter the actual number or change the ratio of mast cells, in any of the salivary glands in hamsters killed 3 to 18 days after irradiation In this irradiated group the average ratios of mast cells in 21 hamsters (11 males 10 females) was 1 2 5 1 for the submandibular

sublingual and parotid glands, respectively. These same glands from 21 controls (12 males, 9 virgin females) had average ratios of 1:2:0.7, respectively.

Burstone[15] irradiated mice by subcutaneous injection of a suspension of radioactive chromic phosphate (containing P^{32}, approximately 15 μc per mouse) adjacent to the left submaxillary gland' and killed the mice 7 days after injection. After fixation by the Altmann-Gersh freezing and drying method he found that the alveoli in both sublingual and submandibular glands stained less intensely by the Hotchkiss PAS method than normally, and attributed this tinctorial change to the depolymerization of glycoprotein. Burstone observed that this decrease in stainability was much greater in the submandibular than in the sublingual gland and, as the result of enzymic tests, he concluded that the submandibular is more radiosensitive than the mucus-secreting sublingual gland.

Low-protein diet. Ten Syrian hamsters were kept 10 to 36 days on a protein-free, otherwise balanced, diet (General Biochemicals, Inc.) to which 2 per cent protein by weight in the form of powdered brewers yeast (Mead) was added with water *ad libitum*, for 10 to 36 days. All 10 showed an increase in the number of mast cells in the sublingual gland. Half of these hamsters had 2 to 6 times as many mast cells in the sublingual as in the submandibular glands. The mean respective ratios for the submandibular, sublingual and parotid glands in these 10 hamsters was 1:3:1; it was 1:2:0.7 in 21 control animals.[6, 7]

The distribution of mast cells in the salivary glands of these protein-low animals approximately paralleled the condition in the control group. About 86 to 90 per cent of the mast cells were contiguous with alveolar cells in the sublingual, and 16 to 25 per cent were adjacent to alveolar cells in the submandibular and parotid glands. The rate of release of mast granules in the submandibular gland of the protein-low hamsters approximately equalled that in the controls.

Pancreas and Islets of Langerhans

Mast cell distribution in the pancreas follows the general plan for similar compound glands (such as the salivary and mammary glands)[181] in which these cells are situated periductally in the loose interalveolar and para-alveolar connective tissue.[181, 31, 53] The pancreas of Syrian hamsters has fewer mast cells than the salivary glands.[531, 35]

The islets of Langerhans appear to be fairly definitely dependent upon mast cells to trigger their activity, for we found a single mast cell in the periphery of an islet in more than 50 per cent of all islets in 6 μ sections of the pancreas of normal hamsters (fig. 10). Thus it appears that mast

cells exert the same type of vascular control over the activity of the islets as they do with the parathyroid glands and with the thyroid follicles (fig 11)

The part played by mast cells in the pancreas is, apparently, primarily related to the regulation of vascularity, whereas this appears to be their only function in connection with the islands of Langerhans The pancreas of normal hamsters has fewer mast cells in the connective tissue near the alveoli than do the salivary glands In point of distribution, the mast cells in the pancreas of hamsters occur in adjacent connective tissue outside of the lobules rather than between the alveoli This distribution and paucity of mast cells may be correlated with the fact that pancreatic juice contains very little MPS compared with the amount of protein, whereas saliva is fairly rich in MPS, but contains less protein than does pancreatic juice This proposed explanation is based upon mast cells as an important source of AMP It does not consider mast cells as a source of histamine, or the relations of histamine and heparin to mast cell induced hyperemia that involves the pancreas

The salivary glands normally produce comparatively little saliva except during mastication of food when production is at its peak [60] The pancreas is, presumably, constantly secreting a maximal amount of pancreatic juice during the passage of chyme from the stomach into the intestine a matter of about 4 75 hours to empty the human stomach of a relatively fat free meal [60] It is also interesting to note that the *per diem* volume output of saliva and of pancreatic juice is about the same 1 to 1 5 liters [60]

Certain Submucosal Tubular Glands

The relation of mast cells to the secretion of mucin occurs not only in the sublingual glands and mucin secreting area of Brunner s glands (fig 13), but also in other mucin secreting glands that are characterized by the presence of very little connective tissue, *e g*, the submucosal glands in the trachea and the mucin secreting alveoli of the mucoserous glands in the tongue and olfactory membranes Thus the mucosa of the oronasal cavity pharynx, e ophagus trachea and gastrointestine is perforated by the ducts of great numbers of small tubular glands which richly contribute to the lubrication of the mucosa Mast cells are more numerous in the mucin secreting areas than in the serous areas in all of these glands The smaller the mucous gland the fewer the mast cells, because the epithelium has ready access to available MPS in the ground substance underlying it and surrounding the mucous secreting glands These small tubular glands characteristically do not extend into the tunica muscularis if it is present, but generally lie in the lamina propria mucosae and submucosa Some of these small tubular glands are purely serous Others are purely mucous and

some are mixed [39, 393, 493, 536, 660] Attention here is directed to the secretory end pieces of these glands rather than to their morphological classification Mast cells in varying numbers always occur in the submucosa and general region associated with these small tubular glands

ORONASAL TRACHEAL AND LACRIMAL GLANDS

Because of the small size of the glands in the submucosa of the mouth and nares one would expect them to obtain their supply of AMP chiefly from fibroblasts as do the goblet cells in the intestine Nevertheless a relationship similar to that of normal salivary glands is indicated by the mucin-secreting submucosal glands in the tongue, pharynx, nares and trachea of normal hamsters The mucin secreting tubular glands show a decidedly greater periglandular concentration and disintegration of mast cells than occurs in the submucosal serous glands in these regions

Eggston and Wolff[270] state that goblet cells occur in certain areas of the respiratory tract notably the posterior surface of the epiglottis' and in the nasal mucosa of man These intraepithelial glands since they "do not penetrate the basement membrane' and are situated entirely within the epithelium[270] should not be confused with the submucosal tubular glands, for they are unicellular or goblet, glands and probably obtain the components for the secretion of mucin directly through the basement membrane

It is claimed that although seromucous (mixed) glands occur in the respiratory tract, they are not found in the nasal mucosa of man [70] Our work does not presume to settle this question, however, we consistently found both serous and mucous glands in the oronasal and tracheal submucosa of hamsters

Other investigators have recorded a high number of mast cells in the tongue[693, 721] and have noted a correlation of high histamine content with an abundance of mast cells in this organ [721]

The subserosal glands of the trachea in hamsters become much larger than those in the oronasal region and also contain a greater number of mast cells These glands attain maximal size in the larynx where they may pass between cartilage and form loose folds or coils in the larger areas of loose connective tissue outside of the cartilagenous plates Mast cells are numerous in the loose connective tissue of the trachea but are notably increased near the submucosal glands

Other serous glands such as the lacrimal gland contain very few mast cells However, the conjunctival mucous glands of Krause in the eyelid contain more mast cells than the lacrimal gland but not so many as occur in the sublingual salivary gland Apparently the glands of Krause secrete some mucus but it is in a dilute form These glands appear to represent a

cross between the purely mucous and the purely serous type of gland Each secreting cell, however, is the same There are no areas that are exclusively mucinogenic or exclusively serum secreting

BRUNNER'S (DUODENAL) GLANDS

We regard the fact that the duodenum has a "high histamine content" which is "associated with an abundance of mast cells"[7 1] as correlated with our[5 8] finding that the high content of mast cells in the duodenum of the hamster has a direct relation to secretion by Brunner's glands We conducted further experiments designed to aid in determining whether or not mast cells supply substances for mucin secreting cells Briefly, the results we obtained indicate that the chief function of mast cells, in addition to their vasomotor relations, is the synthesis and storage of MPS which may serve as an essential component in the production of mucin and certain other substances

The significance of mast cells in mucin secretion is evident in the difference in the number and disintegration of mast cells in mucous as compared with serous, secreting areas of Brunner's glands of the hamster (fig 13) Incidentally, they offer a good site for this comparison because both mucous and serous areas are present in distinct levels in the same section of tissue[5 8]

Mast cells associated with the mucous alveoli were more numerous than those associated with the serous alveoli of Brunner's glands in 10 normal hamsters, 91 to 380 days old in which the mast cells in each hamster were definitely more numerous in the tissue that surrounded the mucous end pieces than in the same situation in relation to the serous end pieces in Brunner's glands[5 8] Localization and disintegration of mast cells in areas of mucous end pieces in Brunner's glands were similar to these relations to the alveoli of sublingual salivary glands Thus, Brunner's glands further indicate that mast cells may provide MPS for the secretion of mucin[5 1 5 8]

Forty nine hamsters (23 males and 26 females) 91 to 382 days old were individually subjected to total body x irradiation (as described under Salivary Glands of Experimental Animals in this chapter) and studied to determine the effect of x irradiation on mast cells and secretion of mucin by Brunner's glands[5 8] Exposure to 400 r decreased the number of mast cells and correspondingly reduced the secretion of mucin by Brunner's glands in 4 out of 8 hamsters that were killed, 3 to 8 days postirradiation Eight out of 9 hamsters that were killed 2 to 8 days after receiving 1200 r had very few mast cells and showed little or no secretion of mucin by these glands Twelve out of 13 hamsters that were killed 6 to 9 days after receiving 995 r, and 7 out of 12 that also received 995 r but were killed on the 10th day after irradiation, had a definitely decreased mast cell popu

lation in the mucosa and submucosa of the duodenum and decreased mucin secretion by Brunner's glands However, 5 others that were killed on the 10th day after irradiation had no detectable change in either the number of mast cells or in mucin secretion The mast cell number and mucin secretion were about normal in 4 out of 6 hamsters that were killed 18 to 33 days after receiving 995 r [5 8]

GASTRIC GLANDS

A review of the available literature indicates conclusively that histamine both exogenous and endogenous, is very effective in stimulating gastric secretion [174 390] but the part played by gastric mast cells as the chief source of the endogenous histamine has not been definitely established Mota and co workers[7 1] obtained relatively high histamine values for the fundic region of the stomach in normal rats However, the administration of compound 48/80 failed to reduce this histamine content significantly and correlated with it, they found few mast cells in normal rats and none in those treated with 48/80 Thus the work of these investigators indicates that only a very small amount of gastric histamine (about 0 3 $\mu g/g$) is derived from mast cells in the rat whereas the total amount found in the fundus of normal rats was 15 1 $\mu g/g$ Douglas and colleagues (1951) obtained histamine values in dogs of over 100 $\mu g/g$ for the fundic mucosa and 68 to 70 $\mu g/g$ for the entire thickness of the fundic wall [301] Feldberg and Harris (1953) obtained histamine values of 140 $\mu g/g$ for the region of the fundic parietal cells[301] which indicates a strong relation between histamine and secretion of gastric juice and acid Although Feldberg states that mast cells were present in the gastric submucosa he expresses doubt that the great amounts of histamine particularly the high value of 140 $\mu g/g$ are of mast cell origin We found a few mast cells in the gastric mucosa of hamsters but they were much more numerous in the underlying connective tissue

Mast cells as a source of gastric histamine as Morton and/or Stavraky (1945 1947 1948) have shown by arterial injections of acetylcholine into the lesser curvature of the stomach may have an indirectly stimulating effect on gastric secretion by releasing histamine which mediates the action of acetylcholine on parietal cells [184] Several investigators have shown that subsequent to section and degeneration of the parasympathetic bearing vagus nerves gastric secretion is stimulated by histamine and that atropine which abolishes secretion in intact animals failed to inhibit the stimulating effect of the histamine in these animals [184] Babkin (1930) and Webster (1931) found that histamine had very little effect on mucin secreting cells in the stomach and had no effect on the chief cells [184] However it stimulated the acid secreting parietal cells and 'thus established that

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positive, water insoluble MPS secretion and the other 10 females which received only 3 to 8 injections of estrogen (1 to 4 mg) during 1 to 77 days did not develop these endometrial cysts[5 9]

Fourteen female hamsters that received 6 to 29 subpannicular injections of progesterone (6 25 to 45 0 mg) during 9 to 26 days and which had previously received 9 or more injections of estrogen were not protected by the progesterone[5 9] Six out of the 14 developed the characteristic, estrogen produced type MPS cysts in the endometrium[5 9]

Permeability to Ions

Another point well worth considering although it is usually associated with muscular function is the importance of potassium in regulating permeability Szent Gyorgyi[100] points out that estrogen and progesterone 'change the ionic permeability in the uterus which may drastically involve the potassium ion permeability which 'is a basic feature of life" and would be expected to influence uterine glands He further points out that 'potassium permeability ' in the uterus is many many times more sensitive to effects of estrogen and progesterone than it is in the heart The point of application at this time is that a continued disturbance of the delicate potassium balance may be the essential mechanism which enables estrogen to initiate a series of permeability changes that result in endometrial hyperplasia with the formation of PAS positive, MPS cysts or carcinoma

Mammary Glands

The primary advantage of trephocytes in many localized tissues appears to be the retention of certain substances within a localized area We have previously pointed out that one primary function of lymphocytes and plasmacytes in wound healing and chronic inflammation is that of bridging the protein gap The same inter bridging effect with regard to AMP pertains to mast cells in several organs such as the mammary gland The mast cells in this tissue appear to have the function of storing AMP from degenerating collagenous or white connective tissue and retaining the AMP in the localized area for one of several purposes such as the detoxication of estrogen or for the subsequent availability of carbon chains for other processes The increase of mast cells in the mammary gland could facilitate a regulatory mechanism for the detoxication of estrogens A number of investigators report the presence of an unusually high percentage of mast cells in the mammary glands in resting or in active stages and that these cells are conspicuously more numerous when they are associated with alveoli and secretory ducts than in the more distant connective tissue[693] At least one investigator Huguenin believes that, developmentally mast cells appear in the mammary glands much later than in the skin[693] Al

histamine acts as a specific stimulator of the parietal cells' of the stomach [184]

Code[175] leaves no room for controversy over the question whether or not histamine in gastric juice is effective in stimulating parietal cells He cites the work of several investigators which shows that in order to produce even very slight secretory response by the application of histamine to the free surface of gastric mucosa, 'tremendous quantities of histamine must be applied' because in the stomach and intestine the surface of the intact mucosa is almost "an impenetrable barrier to the passage of histamine from the lumen Concerning the existence of a chemical mediator, Code shows that evidence is entirely lacking for the existence of a mediator or of a chemical transmitter between histamine and the parietal cells which it is known to affect Thus, for exogenous histamine to cause the secretion of gastric juice, it must be parenterally administered This finding supports the effectiveness of endogenous histamine irrespective of whether it *is plasma borne or liberated from nearby mast cells*

UTERINE GLANDS

Various investigators have shown that the percentage of mast cells in the uterus is extremely high especially associated with the larger blood vessels in the connective tissue, but that mast cells are extremely rare in the mucosa of the uterus [693] We[5 9] have noted the paucity of mast cells in the mucosa and have observed the abundance of these cells in the stratum vasculare in the uterus of hamsters Suomalainen[937] observed mast cells in the uterus of mice

Mast cells are fairly well represented in the decidua, where they have been observed losing their granules and in the chorion but they are sparse in the gravid puerperal and inflamed uteri [693] It is interesting in this connection to note that other investigators report a tremendous increase in plasma histaminase during pregnancy which is attributed to the production of histaminase chiefly by the placenta [504, 507] It has been suggested that this great increase in circulating histaminase prevents the appearance of excess histamine in the blood during pregnancy [07] This would also be expected to account for the paucity of mast cells in the pregnant uterus

EFFECT OF PARENTERAL ESTROGEN

Subpannicular injection of aqueous estrogen (3 0 to 14 5 mg Lakeside) 6 to 39 times during 8 to 77 days into 20 young adult, virgin hamsters did not significantly alter the metachromasia in the endometrial connective tissue nor the number of mast cells in the endometrium or stratum vasculare [5 9] However 10 of the 20 hamsters developed numerous endometrial polypi most of which contained one or more large cysts filled with a PAS

the mast cells underwent cystic degeneration At the same time, others were supposed to have migrated from the outlying connective tissue into the glandular areas so that the number in the connective tissue was diminished while that in the area of the mammary glands was increased [35]

It is thought that the administration of estrogen to male rats (supposedly intact animals) causes hypertrophy of the mammary gland with increased mast cells and modification of the stroma associated with the glands Since these events are concurrent, it is suggested that the mast cells play a part in the modification of the stroma and in fibrillogenesis [3]

though Loeb[61] records the presence of mast cells in the stroma of the mammae in women and rats, he does not relate their presence or number to any stage in the estrus cycle Riley[9] observed chains of Type I (immature) mast cells arranged along muscularly walled blood vessels and scattered Type II (mature) mast cells chiefly associated with capillaries in the connective tissue adnexa of the mammary glands of rats

The increased number of mast cells, following and during the period of resorption of collagen, provides a ready localized mechanism by which excessive concentration of estrogen in the mammary gland may be detoxicated If detoxication of estrogens were dependent entirely upon the liver, the cycles of activity within specialized organs such as the mammary gland and uterus would be expected to be more prolonged if mast cells did not provide sulfuric and glucuronic acids for local detoxication The changes in the mast cell population in the mammary gland illustrate an interesting cycle by which trephocytes can bridge an interval of time and during this process serve several functions in this case, the reabsorption of collagen for subsequent other processes, including the detoxication of estrogens and the increase of the permeability of capillaries and connective tissue ground substance

Changes in the number of mast cells would also facilitate sporadic action of substances such as estrogen For example the effects of a heavy concentration of estrogen could readily be decreased by the detoxication of the hormone by glucuronic and sulfuric acids from mast cells Thus the great number of mast cells in the mammary gland of hamsters could facilitate and increase the speed of the estrogen cycle For example an increase of estrogen in the mammary gland might have a certain prolonged effect, but ready detoxication within this gland could inhibit the initial function of the hormone *Similar relations could* occur in the uterus

Three to 29 injections of aqueous estrogen (1 4 to 29 0 mg) during 1 to 64 days notably increased the number of mast cells (figs 14 and 15) and the amount of collagen in the mammary glands of 19 virgin hamsters 42 to 96 days old as compared with the mammary glands of 24 normal virgin controls of comparable ages These 19 estrogen treated females also had more mast cells in their mammary glands than in the glands of 6 pregnant 5 postpartum or in 5 control hamsters injected with the estrogen vehicle only[530]

In male rats mast cells are said to be fairly numerous in the subcutaneous connective tissue of the body but are much less numerous at the site where rudiments of the mammary gland occur In rats that received heavy doses of e trogen mast cells in the mammary glands had a tendency to increa e soon after the beginning of the experiment Later the number diminished and as the period of estrogen treatment was prolonged ome of

contractility and tonus of the preterminal ("*metaarterioles*") and terminal arterioles and the capillary sphincters that cause vasodilation stasis and leakage of plasma and protein into the intercellular spaces Cortisone prevents and corrects this vascular condition and inhibits transformation of combined (bound) to labile (readily freed) histamine

Certain investigators apparently believe that histamine inhibits wound healing Barnard (1955) suggested that under certain conditions of wound healing failure including general and similar conditions in homografted skin of mice, "healing may be initiated promoted or accelerated by the application of certain antihistamine or other substances all of which are competitive inhibitors of hyaluronidase [361]

Vitamin like functions of histamine have also been suggested The inference is that most if not all vitamin histamine" is absorbed from the alimentary tract and that much of it may be found as conjugated histamine in the urine but that little or none of this absorbed histamine occurs in the urine in a free state [329, 330] The work of Wilson (1954) and others supports the theory that histamine is, or is related to a vitamin [330] Gaddum[330] states that dogs and cats do not make histamine but they absorb it as a vitamin, apparently from their meat diet which as Anrep (1944) has shown for lions and other great cats supplies relatively large amounts of histamine as is indicated by the great increase in the amount of conjugated histamine (probably acetylhistamine) in the total urine histamine [329, 330]

Kahlson (1956) in the discussion of Gaddum's[330] paper challenged the validity of the basis for the theory that histamine may be considered a vitamin His objections were based upon two significant observations (1) Schayer ' has shown that bound histamine has a turnover with a half-life of about 50 days in the guinea pig and (2) he and others have shown that instead of the histamine concentration in the gastrointestinal mucosa being depleted in starved cats (30 per cent body weight loss) it was not altered

SOURCE OF HISTAMINE

Histamine occurs in all animal and vegetable tissues [-39] and is especially high in spinach leaves and flowers nettles tomatoes and lambs quarter[301] and has been extracted from a number of different kinds of fungi [744] Histamine occurs chiefly in an inert form[86, 171, 407, 416, 855, 869, 900, 933, 104, 1048, 1134] and in stages in which it is in combined labile or free forms [290] Various investigators indicate that histamine occurs in essentially all cells [239, 417] including most plant cells [-39] or at least in all animal tissues [45, 1094] However Gaddum[3, 9] states that most of the extractable tissue histamine seems to be contained within the mitochondria or other

5

Histamine

The importance of mast cells as a source of histamine and heparin has opened a wide field of approach to the investigation of several pathological conditions associated with the disturbance of the orderly processes involved in maintenance and/or growth processes The basic principles involved are primarily the varying degrees of anemia or hyperemia and of capillary permeability changes in response to quantitative release of mast cell products

Mast cells have been shown to be a significant, if not a major, source of histamine[65, 135, 297, 366, 811, 814, 856, 1085] They have an estimated content of 7 to 32 μg of histamine per cell in man[366] and according to some investigators, have reached 950 μg of histamine to 1 g of tissue in a large solitary lesion in a human case of urticaria pigmentosa[1085] The all time record *for these investigators was 1290 μg of histamine to 1 g of tissue from* a mastocytoma of a dog Several tissues that are high in histamine content also have a high number of mast cells[65, 366, 711, 831, 856, 857] however, histamine is not present in embryos until the appearance of mast cells which occurs near parturition[1085] The histamine content of mast cell tumors varied from low values to 1290 μg/g in a particular mastocytoma[1085]

Mast cell granules have been credited with being an important source of heparin[500, 811, 854, 1085, 1094] This assigned function does not obviate the fact that histamine[6, 297, 366, 374, 457, 665, 797, 851, 855, 1085] other substances, possibly including serotonin,[6] and a so called spreading agent[666] may also be present in the cytoplasm between and/or within the granules of mast cells[6, 97] These substances in addition to histamine and heparin may be important for the correct interpretation of the functions of mast cells

Mast cells however are not the only source of endogenous histamine, for it has been shown that histamine is present in leukocytes, especially *in* the buffy coat of centrifuged blood in blood platelets of certain species, and, to a certain extent, in all mammalian tissues and body fluids Intracellular histamine is in a bound or inactive storage form[45, 301, 416, 900, 1005]

Histamine probably has multiple, indirect physiological effects but its best known direct effect is the production of vasodilation accompanied by increased capillary permeability Halpern[390] believes that especially in inflammatory processes, histamine as *well as free metabolites, inhibit the*

of the blood supply to the ear of a dog or to the superior limb of either dog or man. Similarly, using gastric secretion, bronchiolar tone and arterial blood pressure as indicators they were unable to detect an increase in histamine following brief occlusion of the postrenal abdominal aorta by clamps in cats and guinea pigs. The accuracy of any tests for plasma histamine content is open to question since it has been pointed out that blood tests for experimentally produced local histamine are usually negative, because the released histamine rapidly diffuses out of the blood and/or is destroyed by enzymes.[70]

SPECIES DIFFERENCES

There are species differences in the histamine content of blood. Tarras-Wahlberg reported that the blood histamine content of the rabbit is 2000 times that of the cat.[119] The whole blood histamine content, as shown by several investigators, is probably higher in the rabbit (1 to 5 μg/ml) than in any of the more commonly used mammals. It is lowest in dogs and cats (0 to 0.04 μg/ml). The blood of man (an omnivore) has a histamine content (0.02 to 0.08 μg/ml[1,4] 1 to 2 μg free histamine/liter of plasma)[3,9] intermediate between that of rabbits (herbivores) and that of cats and dogs (carnivores).[174] However, blood histamine content cannot be definitely related to diet, for blood of the phylogenetically queer, daily ascorbic acid requiring guinea pig has a histamine content (0.06 to 0.8 μg/ml)[174] more nearly comparable to that of man than with that of the rabbit. As far as we know a serious attempt has not been made to explain why or how these species differences in blood histamine content occur. It would be interesting to know whether certain mammals, such as cats and man, have less production or greater destruction of histidine, which is generally believed to be the source of all histamine,[391, 399, 900, 993] or have a greater destruction of histamine than rabbits during a 24 hour period. It is quite possible that rabbits are more economical than cats and man in the rate of production and release of histamine, because the blood platelets of rabbits are rich in stored histamine.[80] Rabbits appear to have fewer and smaller mast cells than hamsters, rats or mice, but it is not known how the mast cell population in general compares in rabbits, cats and dogs.

The amount of histamine that normally occurs in the blood plasma is related to that found in the various regions and structures of the body—to its endogenous and exogenous sources, release and degradation. Thus histamine is found under normal conditions in the bile, gastric fluid, nasal secretions, sputum (usually not in saliva) and urine, but it has not been reported to occur in pancreatic juice.[301, 3, 9] The blood plasma content

particles of the same size,' as indicated by centrifugation Other investigators support this view by stating that the histamine within the mitochondrion like particles is probably in a diffusible form[374 6 7]

Histamine is an essential, widely distributed and physiologically very active substance[60 76 416 1084] The local and/or systemic release of it must be adequately controlled, as is shown by allergic reactions which are generally believed to be related to the explosive liberation of histamine caused by the entrance of the sensitizing substance into the tissues'[1084]

The distribution of histamine varies widely in adults of different species[301] and in different organs in a single species Feldberg[301] shows that the average histamine content of the liver varies from only traces in the guinea pig to less than 1 µg/g in rats, whereas extremes of 8 to 110 µg/g have been found in dogs and horses and also very high values in rabbits Benditt and collaborators[6] conclude that the histamine content of the areolar, 'subcutaneous' tissue from the back and from the skin on the dorsum of the feet "is related to its mast cell concentration in the rat Riley[8 0] found that four mast cell tumors contained a great amount of histamine and concludes that 'there thus appears to be some grounds for the belief that not only do tissue mast cells contain heparin they are also rich in histamine' His and West's[8 7] more recent work confirms this point Study of regions of dog skin and lung show that regions rich in histamine are also rich in mast cells[366]

It has been conclusively established that histamine is readily produced by decarboxylation of histidine,[60 407 41 416 993 1048 1084] similarly by bacteria in the intestines[330 993] and by a cyanide sensitive enzyme which has been isolated from the liver and kidneys of guinea pigs rabbits and rats[416] Schayer[900] believes that all the histamine bound in the organism arises from histidine'

Plasma Histamine

A small amount of free histamine occurs in blood plasma, but most of it is stored in an inactive or bound form presumably with a protein, within the cells and when released enters the tissues and circulating fluids where it becomes biologically active by being bound again on 'specifically histamine sensitive" effector cells[86] Various investigators have reported that venous blood contains an increased amount of histamine during the period of hyperemia following a temporary (up to 20 minutes) occlusion of the flow of blood to a limb or to resting skeletal muscles in dogs and man[85] Conversely, Emmelin and co workers[28] using Code's modification of the Barsoum Gaddum method were unable to detect any increase in venous blood histamine following brief (10 to 20 minutes) obstruction

from metabolism is conjugated but about 30 per cent of that derived from oral administration is conjugated

Storage of Histamine

There is considerable diversity of opinion concerning where and how histamine is synthesized, inactivated and stored The work of Blaschko [86] Schild[904] and others indicates that most of the cellular content of histamine, as well as other biogenic amines is stored by being bound to intracellular cytoplasmic granules (mast granules large granules and mitochondria)

Schayer[800] apparently believes that in rat skin, histamine is stored as histamine' in little binding sites or cubicles which will just hold one molecule of histidine instead of being retained in a hypothetical bag The cubicle is permeable to the molecule of histidine which after entering is decarboxylated and the histamine retained in that same site McIntire (1956), believes that preformed histamine 'held by very labile bonds is stored in abundance in the tissues [1045]

Most of the histamine in the blood, apparently is bound to the white cells[174] and platelets [86] A number of investigators[174] found comparatively little histamine in the erythrocytes and plasma They also found that 70 to 100 per cent of the total blood histamine occurred in the leukocytes (the white buffy coat of centrifuged blood) of bullocks dogs goats horses and men As a result of work done with several types of histamine liberators [1045] values somewhat different from those cited by Code[174] are deduced Code points out that during the clotting of blood the histamine is forced out of the white cells into the serum He also states that lymphocytes lymph nodules, monocytes and platelets contain little or no histamine [174] but this statement is probably in error as regards platelets because of the technique he used [1045] Dale (1955) appears to agree with Ungar s[1045] criticism of Code s tenet as applied to the rabbit for he states that rabbit platelets contain a whale of a lot of histamine Those of the horse contain no histamine at all [86]

Halpern[390] has a different approach He suggests that histamine is stored in the living organism in three forms (1) combined histamine which can be freed only by destruction of the cell (2) labile histamine which is freed by action of histamine liberators physical chemical traumatic and other generally accepted means and (3) free histamine the small quantity which may be found in free form and is constantly effective It requires several hours or possibly several days for the combined form to become the labile form whereas the labile form can transform into the free form in a matter of minutes [390]

may be rapidly and excessively increased by administration of substances or under other conditions which suddenly release large amounts of stored histamine [3 9]

HISTAMINE IN THE URINE

The amounts of free and conjugated histamine in the urine are fairly reliable indicators of its source [330] Thus, the amount and condition (whether free or bound) of urine histamine should help to explain some of the quantitative variations observed in plasma histamine Gaddum[339] shows that conjugated histamine and a small amount of free histamine' appeared in the urine following the oral administration of histamine to dogs However, only free histamine appeared in the urine of dogs that received injections of histamine parenterally He points out that meat which contains histamine, produces high concentrations of conjugated histamine in the urine when fed to carnivores, specifically lions and tigers The urine of elephants and horses, however contained only ' small amounts of free histamine and none in the conjugated form " In this connection it would be interesting to know what percentage of the blood histamine was conjugated, or what percentage was free and if the ratio of conjugated, or of free histamine to the plasma histamine level differs significantly in the cat and rabbit

Anrep and co workers (1944) contribute to the answer to this question by stating that the concentration of free histamine in the urine of rabbits is less than 100 μg/l and the total quantity of histamine, including both free and conjugated, is variable in the cat dog man and rat, the con jugated histamine in the urine of the cheetah leopard, lion and tiger was greater than 3000 μg/l [3 9] Wilson (1954) confirms previous reports that the rat excretes about 0 5 μg/g of body weight during a 24 hour period, which is about 125 μg per day in the urine of an adult 250 g rat [330] Most of the histamine in the rat urine was free since these investigators and others report that only about 13 per cent was conjugated [330]

It appears that within a certain range of dosage the major part (by far) of histamine that is administered parenterally to rats is destroyed or elim inated by routes other than the urinary Wilson (1954) injected histamine by different routes into rats and estimated that about 14 per cent of the amount injected appeared in the urine [330] He also reported that injected histamine increased the amount of free but did not affect the amount of conjugated histamine in the urine of rats When the histamine was ad ministered by mouth it greatly increa ed (more than doubled) the amount of conjugated, but only slightly increa ed the amount of free histamine in the urine of the rats [330] Very little of the urine histamine that results

a form of bond between them of an ionic and/or secondary valence character

"The interrelations of a macromolecular anionic substance, such as heparin, with histamine which is cationic can involve a combination of factors including the electrostatic attractions of salt ions hydrogen bonding forces hydration, and possibly Van der Waals forces The disruption of any one or more of these factors or forces may facilitate the release of histamine "The great diversity of influences elicited by the many different substances and treatments that can effect the release of histamine rule out even a remote possibility of their exerting such a common effect as would be necessary to promote a possible enzymatic release of this compound The release of histamine by enzyme action has never been confirmed experimentally

The investigations and views of McIntire[670] Riley[835] MacIntosh,[6 7] and their respective co workers strongly support the views I have expressed above

Release of Histamine

The release of mast cell granules by experimental means is accompanied by the release of histamine It has repeatedly been shown that injection of histamine liberators, such as compound 48/80[457 7 1 7 8 4 8 7] stilbamidine[7 8 1 856 857] and ovomucin[6] burst or explode the mast cells of rats anti hamster serum caused the granules of the mast cells of hamsters to rupture and scatter the granules throughout the tissue[1078]

HISTAMINE LIBERATORS

The mode of action of histamine releasers is obviously not clearly understood and to make the problem more complicated histamine is present in more than one form and in more than one type of cell or situation and it is released by almost any injury or damage to tissues[426 8] including the effects of x rays ultraviolet or actinic solar rays electric current frostbite and burns[416] Paton (1956) states that DeKanski (1945) found that within a few (15 to 30 or more) minutes after injuring mice by burning the total body histamine was doubled[330] Gaddum[330] suggests that in this experiment the surprising amount of liberated histamine was apparently formed in the skin of mice It is also believed that certain substances are capable of liberating histamine from one cell but not from another Thus as Perry (1956) points out compound 48/80 explosively liberates histamine from mast cells but is quite incapable of freeing histamine from blood platelets[164] To establish a semblance of order in this chaotic situation Paton[78] groups all the various substances and agents that are capable of releasing histamine into seven classes one of which he desig

Another important thing to take into consideration in connection with histamine storage, as pointed out by Perry,[797] is that some of the histamine liberators are capable or releasing histamine from certain cells but not from others Thus, compound 48/80 is held to be 'fairly specific' for mast cells,[85] but probably incapable of freeing histamine from other cells such as the blood platelet[86 104] which, by use of the antigen antibody complex or other liberators, has definitely been shown to contain histamine[1045] Riley and West[857] support this idea by considering the mast cell as "the target for compound 48/80 " They point out that "the mast cells and the histamine disappear together" following the administration of compound 48/80

The binding and inactivation of histamine serves to control one of the most physiologically active substances produced in the body[45 416 417 1084] Histamine may cause a variety of changes in the tissues[407 41 1048] and structures However, the production of hyperemia as a result of capillary dilation and consequently, the greatly increased capillary permeability,[60 00 41? 390 391 416 0] and the subsequent escape of protein rich plasma[60] are most significant from the point of view set forth in this work

There are several ideas on the mechanism of histamine binding, but the experimental results are not conclusive Histamine is believed to be controlled by being bound to basic protein[407 416] Rocha e Silva (1946) believes that it is bound by peptide linkage to a carboxyl group," probably in lysine or arginine,[3 9] or to certain amino acids in the cell[407] Lecithin[3 9] is also believed to bind histamine in an inactive or ineffective form[45 135 390 391 407 416 6 0 869] The work of several investigators indicates that enzymic action, such as that required to break protein linkage is far too slow to account for the often observed sudden to explosive release of histamine[8 9] Another mechanism considered is by ion exchange[785] by ionic binding in cells[669] or by segregation in mitochondria or mitochondria like particles[330] so that the histamine may be released as needed

Clopton[171] summarizes the general problem of histamine storage from a chemical point of view Current recognition of the fact that mast cell granules represent the principal source of heparin and histamine has led to much speculation regarding the chemical and physical relationships of the e two physiologically active substances Of the various theories proposed, experimental data strongly support those based on the presumption that histamine is held in loosely bound form as the salt of an acidic substance such as a protein or a large molecule of the heparin type Histamine of mast cells can be relea ed without the mediation of an enzyme and is not held by a peptide bond

The correlation of histamine and heparin content of mast cells suggests

ator or an anaphylatoxin "[374] Fawcett [97] found that the injection of compound 48/80 caused the mast cells to release their granules and large *amounts of histamine Although he contends that mast cells are unusually* rich in histamine, it is not clear whether the histamine, or histamine precursor (histidine), is in the cytoplasm or within the granules

Mota and co workers[721] *suggest that although mast cells are very susceptible* to 48/80, this compound is unable to release histamine from some tissues "due to the fact that in these the histamine is not of mast cell origin " *They believe that the inability of compound 48/80 to release* histamine from cells other than mast cells may explain the conclusions of other investigators using this compound, that certain tissues such as those *in the wall of the gastrointestine do not contain histamine This view is* strengthened by West s[1035] statement that his and Riley s studies leave no doubt that mast cells are exceptionally rich in histamine and that in many *instances the total histamine content of an organ can be accounted* for in this way " However, they do not claim that mast cells hold all the histamine in the body or that "the production of histamine and heparin are the only functions of mast cells "

It has been explained that the first injection of compound 48/80 produces intense shock and that the tolerance of rats to repeated increased dosage is because *the first dose practically destroys the mast cell population* so that little or no histamine is available for liberation by succeeding doses of the histamine liberator Riley and West[857] conclude from a series of experiments that histamine is stored *in the mast cells and* therefore that the histamine reserve is depleted when the mast cells are ruptured, and consequently additional administration of increased amounts of the histamine liberator is ineffective, as indicated by *its inability to* cause other seizures of histamine shock

The mesentery of rats that received chronic intraperitonal injections of compound *48/80* showed hyperplasia of the milk spots and adventitial tissue although all of the mast cells had been destroyed or reduced to "ghost cells and none had reappeared [857] Mast cells rapidly recover from *the depleting effects of the histamine liberator 48/80* Within 4 days *after* cessation of the application of this compound the mast cell pattern in the skin of the ear and even more so in the subcutaneous connective tissue *was nearly restored* [857] *It has also been shown that with the return of the* mast cells there is a reappearance of histamine in assayable quantities [857]

It has been shown that anti test animal sera will cause mast cells to release *their granules Thus rabbit anti hamster serum injected into hamsters* caused the mast cells to rupture completely and scatter the 'granules throughout the tissue ' [1076] A number of foods known to produce allergic reactions in man *have been shown to be active histamine liberators*

nates as "the histamine liberators" and to which he assigns compound 48/80 and the dibasic and polybasic histamine releasing compounds Paton states that this group of "histamine liberators' probably frees basic histamine which *is associated with some complex tissue acid,"* presumably ribonucleic acid (RNA), by ion exchange Ungar[1044] thinks it likely, especially in inflammation, that histamine liberators act to form a proteolysin by converting a precursor, probably profibrinolysin, to fibrinolysin which then attacks *"certain proteins to which the mediators are bound*

Some of the known histamine liberators appear to be limited in effectiveness to certain cells Others appear to have a more or less general action on two or more, possibly all, cells Perry (1956) calls attention to the fact that the antigen antibody complex will release histamine from platelets and probably other cells whereas compound 48/80 is not known to release histamine from any cells other than mast cells[7, 1, 104] Another interesting point, brought out by McIntire (1956), is that the antigen antibody reaction has a "very significant latency" between it application and the onset of histamine release whereas compound 48/80 and chemical liberators generally have an explosive effect[104] Histamine liberators may also liberate heparin[297, 1085] as has been shown to be especially the case in mast cells[1, 854, 8, 7, 1085] However, some of the smaller supposedly younger or Type I cells of Riley,[8, 1] especially those in the lymphoid milk spots in the omentum and in the intestine, were not ruptured by compound 48/80[457] In this connection it is interesting to note that in rats killed by rapid (5 seconds) intravenous injection of a fluorescent histamine liberator (stilbamidine) Riley[851] found that mast cells appeared in the omentum chiefly in the milk spots in some of which practically every mast cell had concentrated stilbamidine as shown by distinct fluorescence, others often in an adjacent milk spot, may have shown little or no fluorescence He states *that the fluorescent mast cells* were mature *(Type II) cells and suggests* that the non fluorescing mast cells in the milk spots were the Type I, or even younger, mast cells[854] Riley and West[857] suggest that mast cells which lie in the perineurium of peripheral nerves and those in the sub*cutaneous connective* tissue and in the ears are the most resistant to a subacute, repeatedly incremented dosage in rats, of intraperitoneally injected compound 48/80 Likewise West[1085] reports that intraperitoneal injection of ammonia into normal rats greatly reduced the number of mast cells in the mesentery, but did not affect those in the "subcutaneous tissue' Other investigators[7] found that compound 48/80 ruptured mast cells as readily *in vitro* as *in vivo* In addition to these effects compound 48/80 also altered the permeability of cell membranes and the particle cytoplasm interface" of the granule surfaces of the mast granules so that histamine diffused out as a result of the 'basic chemical *histamine liber*

forms of heparin and histamine are contained at the same time in the mast cell[1, 6, 665, 6, 7, 763, 784, 831, 843, 8, 8, 8, 7, 867, 869, 1054] However MacIntosh[6] cautions that it has not yet been established that both heparin and histamine are contained in a single mast cell granule Even if both heparin and histamine do occur in the same mast cell granule the two variously antagonistic substances may occupy different regions in the granule and thus be separated spatially The concomitant storage of these two substances in mast cells would theoretically afford a means of local control of histamine,[135] *since heparin is known to be unable to prevent the release* of histamine in blood cells[416] and that these two substances are antagonistic in other ways[6, 7] Also it has been shown that heparin inhibited the effect of histamine on the contraction of smooth muscle and that histamine inhibited the anticoagulant activity of heparin[6, 7] MacIntosh[6, 7] cautions *that in the heparin histamine linkage the bond would probably be* so weak that the heparin would be incapable of binding the histamine within the mast cell but he admits that if certain conditions were to ob*tain this would be a possibility*

If both histamine and heparin were included in a mast cell hormonal control especially adrenal[9] should be highly efficient Furthermore heparin may inhibit the release of histamine in blood cells[416] and histamine may oppose heparin in stimulating coagulation and in contraction of isolated intestine of guinea pigs[6] Since both histamine and heparin in inactive form and other AMP occur in the mast cell this cell is one of the most highly reactive physiological cell units in the body

Further evidence of the occurrence of both heparin and histamine in mast cells is afforded by the fact that histamine liberators free heparin as well as histamine[8, 4, 105] and that most independent investigations of the origin of each of these two substances show that the mast cell is a rich source of both heparin and histamine[8, 4, 108] West[1050] (fig 1) points out that the liver capsule (Glisson's capsule) of the ox which is said to have about the greatest number of mast cells of any structure[00, 1094] also has a high content of histamine[414] as well as an extremely high content of heparin[00, 1094] Several investigators have correlated the histamine content with the mast cell population of tissues[65, 386, 851, 854, 108] Others have *likewise correlated the heparin content with the mast cell content in the* same or in different tissues[500, 1094] Anfinsen[1] points out that administration of anaphylactogenic agents is believed to release both heparin and histamine from mast cells in dogs

SEROTONIN COMPARED WITH HISTAMINE

The discoveries that serotonin occurs within and is synthesized by mast cells[6, 5, 6, 884] that mast cells also contain histamine[63, 7, 1, 83, 8, 6]

Paton and Schachter (1951) showed that histamine liberated from the skin by compound 48/80 may provoke redness, itching and edema in the form of giant wheals (angioedema or urticaria) in the dog, cat and rat which resemble this condition in man[896] Various investigators have shown that eggwhite contains a substance which actively releases histamine in the cat, and another substance which is equally effective in the rat[896] Strawberries, eggwhite and shellfish are ome of the more common foods which are notorious for their ability to cause giant wheals and the attend ant syndrome in susceptible people Schachter[896] points out that, although the mode of action is not known, there is evidence both for and against the involvement of the antigen antibody reaction

Another aspect to consider in evaluating the relation of histamine to mast cells is the ability of polysaccharides to activate histamine Rocha e Silva[867] states that polysaccharides such as agar insulin and starch, ac tivate the histamine releaser in blood Thus it may be surmised that dis ruption of mast cells may release a form of mucopolysaccharide (MPS) which in turn activates histamine in various cells so that it is free in the body fluids and thereby accounts for some, if not the major portion, of the increase in histamine

Investigations have been conducted with various histamine liberators which apparently result in variations in the ratio of the amount of water to the amount of protein leakage due to capillary dilation Thus Halpern[391] reports that intravenous administration of polyvinylpyrrolidone into dogs produced capillary dilation with an increase in protein leakage of 5 or 6 times normal but leakage of water was only doubled This may be roughly correlated with the observation that certain histmaine releasing substan ces, such as intravenously injected dextran produced vasodilation, pros tration and edema in rats[391] So far investigators of histaminic effect have paid comparatively little attention to the ratio of protein to water in ex travasated plasma

LIBERATION OF BOTH HISTAMINE AND HEPARIN

The occurrence of both histamine and heparin in a single cell may be of primary significance in re olving the controversy over the composi tion and function of mast cells It is claimed that mast cells store and probably synthesize histamine or its components and serotonin, as well as heparin hyaluronic acid (HA) and other acid mucopolysaccharide (AMP) Thus the results of those studies in which it is stated that mast cell granules release histamine may be quite correct without militating against the abundance of histochemical evidence showing that mast cells synthesize and store AMP

A considerable mass of evidence is available to indicate that inactive

serotonin from that of histamine since some of these methods, especially those employing uterus or intestine commonly have been used to detect the presence of histamine[78, 400a, 595, 964] Even though serotonin regularly has pronounced spasmogenic action on certain prepared isolated smooth muscle structures, e g segments of carotid artery,[79, 1117] uterus of guinea pigs, mice or rats[309, 331, 837, 1117] intestine of guinea pigs[837, 931] the colon of rats[65] and heart of *Venous mercenaria*[340, 1035] this reaction of smooth and cardiac muscle is far from being specific for serotonin For example acetylcholine 1 5 million, histamine 1 5 million or barium chloride 1 500 000, in Locke Ringer solution is each spasmogenic for isolated guinea pig uterus or ileum[931]

The uterus of mice sensitized with egg albumin contracts *in vitro* when eggwhite is added[309] However it is believed that isolated strips of normal or sensitized uterus is 1000 times more sensitive to serotonin than it is to histamine[309, 541] In this connection it is interesting to note that injection of mice with *Hemophilus pertussis* cells produced increased sensitivity to both serotonin and histamine[341 7 7 6]

Erspamer (1953) recommends using the atropinized uterus of sprayed rats after the administration of strongly physiological doses of estrogenic hormones affords test material which is very sensitive to serotonin (in dilutions up to 1 200 million) and is negligibly sensitive to histamine[771] This method appears to offer a reliable means for differentiating the spasmogenic action of serotonin from that of histamine However as has been pointed out for other techniques[1040] the possibility exists that the effects of related substances such as certain analogues may be indistinguishable from the effects of serotonin

Another interesting point that pertains to the specificity of the effects of serotonin is that although all antihistamines apparently are not capable of blocking the spastogenic effects of serotonin on smooth muscle diphenhydramine and tripelennamine do prevent the induction of spasm in preparations of isolated guinea pig ileum by acetylcholine and histamine as well as by serotonin[831] However attention should be directed to the finding that the administration of antihistaminic drugs failed to inhibit anaphylaxis of isolated uterine strips or in the intact mouse[308]

Several factors probably affect the results obtained by using smooth muscle but the physiological state of the tissue being used is probably the one most often overlooked Erspamer[59] states that the uterus of rats in estrus apparently is the best test object for the presence or assay of serotonin others[1113] advocate pretreatment of rats with estrogens to insure uterine response Various investigators[866] have shown that the sensitivity of the uterus of the rat to serotonin is high during estrus and in pregnancy but diminishes during diestrus immediately following parturition

[857, 1085] and that serotonin is not formed from 5 hydroxytryptophan by platelets, but is probably transferred to the platelet during its formative period,[1039] whence it may be released[8, 7, 8, 6] along with histamine,[65, 86, 1066] further complicate the problem of differentiating serotonin and its activities, from histamine and its activities [900a] Species differences in response of a structure to serotonin may be taken as an indication that results of biological assay of the structure in question may have only relative value For instance, it has been shown that strips of the carotid artery of cattle are very sensitive to serotonin, but similar strips of this artery from swine and dogs are so nearly insensitive to his hormone as to render the porcine and canine material undesirable for biological tests [771] Small amounts of serotonin or of histamine increased the coronary flow when injected into the coronary artery of the dog, whereas an extract of the neural hypophysis reduced it [909]

So far, it appears that the two amines are much more reliably differentiated chemically and physically than by their pharmacological activities which appear to differ chiefly in potency if smooth muscle is used for the assay [308] Although serotonin (5 hydroxytryptamine) and histamine (5 imidazoleethylamine)[694] are both amines and are both produced in nature by decarboxylation [114, 330, 899, 900, 1040] and although they are recognized as different chemical entities and are usually accorded opposite pharmacological effects the possibility of mistaking the effects of one for the other exists

Tests for Serotonin

Certain investigators have employed the following methods to test for serotonin (1) fluorescence,[114, 933, 1040] (2) a specific chemical method,[1039] (3) electrophoresis [1035, 1037] (4) blood pressure [907, 1115] (5) motor activity of the bladder,[86, 90, 3, 1] (6) gastric juice production and/or bronchiolar tone,[86, 3, 1, 483, 907] (7) nictitating membrane of the cat,[837] (8) kidney of the cat [15, 837] and (9) perfused isolated rabbit ear preparations[15, 770, 830] and other procedures However, the most commonly used method is a form of biological assay of extracts that employs smooth muscle such as rabbit's ileum,[830] but usually the guinea pig's ileum, [86, 3, 8, 342, 483, 831, 1040, 1115] colon strips of the rat,[6] the uterus of rat or guinea pig [152, 90, 8, 7, 866, 1115, 1118] or a muscular artery [79, 15, 8, 7, 837, 1115, 1116, 1119] The isolated heart of a marine clam (*Venus mercenaria*) is recommended as being very sensitive to serotonin[1035] and has been successfully used by others [771, 881, 108] The tracheae of cats and the auricles of rabbits have also been used [941]

The biological methods employed by some investigators for the identification of serotonin apparently fail to differentiate sharply the action of

enzyme which is similar to if not identical with monoamine oxidase and it catalyzes the metabolism of serotonin.[1040] When serotonin was aerobically incubated with homogenates of either kidney or liver, about 30 per cent of the metabolized hormone was recovered as 5-hydroxyindoleacetic acid; the other 70 per cent was unidentifiable as any known compound.[1039] Similar results were obtained *in vivo* in which 20 to 30 per cent of the serotonin administered to dogs was recovered from the urine as 5-hydroxyindoleacetic acid in addition to several indoles which were not identified.[1039]

Since both serotonin and histamine are excreted chiefly by the kidneys, the urine content bears a fairly definite relation to the intake of these amines. This relation is much more significant for histamine than for serotonin since both free and parenterally administered serotonin are rapidly inactivated through oxidative deamination by monoamine oxidase. Very little of the parenterally administered histamine is degradated. The result is that the amount of free urinary histamine bears a relation to the amount of free histamine administered.[339] Likewise, the amount of conjugated histamine in the urine is related to the amount that was metabolized.[330] However, a considerable error may be introduced into this evaluation by certain hypertensive renal vascular conditions, especially with regard to the relation of the amount of serotonin metabolite to the intake. It has been observed that chronic glomerular nephritis or advanced renal disease of malignant hypertension reduced the output of urinary 5-hydroxyindoleacetic acid in man.[93]

Another probable source of error lies in the fact that there is a considerable amount of evidence indicating that the synthesis of both serotonin and histamine is inhibited by a deficiency of pyridoxine (pyridoxine phosphate, vitamin B_6). This effect is not specific for either substance, for pyridoxine appears to play an important part in decarboxylation of amino acids in general and for metabolism of epinephrine and norepinephrine,[1040] as well as for decarboxylation of 5-hydroxytryptophan and histidine.

Acetone extracts of urine from normal dogs and men contained 0.1 to 1.0 μg/ml of serotonin (which is comparable to the 0.1 to 0.4 μg/ml serum content of heparinized dogs). Venous infusion of serotonin increased the urine content of this substance.[103] However, the normal daily output is astonishing, for it has been estimated that normal dogs excrete about 2.0 to 3.0 mg and human subjects about 7.0 to 10 mg of 5-hydroxyindoleacetic acid per day.[109, 1039] These amounts presumably account for most of the dietary values for daily output indicates that the turnover of serotonin in tissues is probably much larger than has been suspected.[109]

The urine of dogs treated with serotonin yielded 20 to 30 per cent of the administered dose in the form of 5-hydroxyindoleacetic acid, the

and after ovariectomy, Woolley[1115] states that "the uterus will not respond to serotonin except during estrus" These objections are apparently overcome by Twarog and Page[1035] who recommend the use of the isolated heart of *Venus mercenaria* to provide a selective and highly sensitive means of assaying serotonin activity

Immersion of a section of guinea pig ileum in a strong solution of tryptamine, 5 hydroxytryptamine or substance P causes contraction in the piece of intestine, but this contraction ceases in a few minutes in spite of the continued presence of a potent amount of the excitant in the solution After the piece of intestine has been desensitized by excess of either tryptamine or 5 hydroxytryptamine, it is insensitive to both substances, but remains sensitive to other stimulating substances[3, 8] This reaction in interpreted as indicating that the stimulating effect of tryptamine and 5 hydroxytryptamine is mediated by one type of receptor in the intestine and that some other substance, such as histamine, causes contraction of the ileum by stimulating other receptors Nevertheless, there apparently is no adequate method of bioassay by which intestinal contraction caused by serotonin can be differentiated from that caused by histamine or certain other agents

Metabolism of Serotonin

Sjoerdsma and co workers (1955) found that the enzyme monoamine oxidase apparently catalyzes the metabolism of serotonin[1040] Other work shows that serotonin is rapidly metabolized by monoamine oxidase when serotonin is liberated in reasonable amounts[114] or administered parenterally[1038, 1039, 1040] That histamine is similarly destroyed by the enzyme histaminase is generally conceded with few exceptions[13] but whether or to what extent diamine oxidase and various other factors are involved is still controversial[507, 899, 100, 1134] Schayer[899] recognizes three kinds of mammalian enzymes which metabolize histamine in physiological amounts These are an acetylating enzyme diamine oxidase and histamine metabolizing enzyme II ' which is probably a composite of an oxidative and a methylating enzyme[899] These enzymes have varying degrees of efficiency in different animals[899]

Urinary Serotonin and Histamine

Since free serotonin exogenous as well as endogenous is rapidly metabolized,[157, 1038, 1040] by far the major part recovered in the urine is in a degradated, inactive state usually in the form of 5 hydroxyindoleacetic acid[1039, 1039, 1040] primarily as a result of metabolism of the serotonin by oxidative deamination[1038, 1039, 1040]

The kidneys and liver appear to contain significant quantities of an

in which they isolated substances from normal human urine which they considered methylated derivatives of serotonin[760] In addition to traces of bufotenin (dimethyl serotonin) occurring in human urine methylation of serotonin has also been reported in plants certain invertebrates and a toad[1040]

It is generally conceded that after the administration of histamine releasing agents[595] or the administration of histamine,[899] there is an increase in urinary histamine[330] and it has been shown that this urinary histamine is spastogenic for atropinized guinea pig ileum[595] Also the works of McIntire and co workers (1947 Rosenthal and Tabor (1948) and Millican and associates (1949) show that after separation by McIntire and co workers method for other urinary substances histamine is the only remaining spastogenic substance acting on atropinized ileum of the guinea pig[595]

CONTROL OF HISTAMINE

The control of the amount of histamine liberated at any one time or place is of great importance because it is a vasodilator substance[1057] that is active in very small quantities and is widely distributed in the organism In considering methods for the control of histamine the following points particularly the origin and storage of histamine are well worth taking into consideration Schayer[900] believes that all the histamine bound in the organism arises from histidine and that exogenous histamine is not picked up by the tissues Investigators are not in agreement on the question of how intracellular histamine is bound or on how securely it is bound McIntire[860] suggests that all intracellular histamine is held by a weak probably ionic type of linkage which should not require the action of an enzyme for the release of histamine

It is generally understood that histamine decarboxylase produces most if no all of the histamine from histidine[899 900 993] Histamine is produced in the tissues at the site where histamine is bound[900] in the kidneys where much of it is excreted rapidly into the urine at least in rats[900] and in the intestine where it is formed by putrefactive bacteria[330 649 993] presumably by decarboxylation of histidine[993]

Mast cells are a source of both histamine and heparin This is highly significant since when freed simultaneously these substances are somewhat antagonistic and thus normally tend to counter balance the effects of each other partly through the vasodilator properties of histamine[1057] and vaso con trictor properties of heparin[386] Eosinophils may also antagonize the effects of histamine and it appears that the antagonistic relations of the eosinophil to products of the mast cell form an important contribution to the maintenance of a proper histamine level The interrelations of epi

urine of those that received 5 hydroxyindoleacetic acid yielded unchanged nearly the entire amount that was administered[1038] From these values the authors calculated that in human subjects about 20 mg of serotonin were metabolized daily to produce 7 mg of 5 hydroxyindoleacetic acid daily[1038]

The urine of large carnivores including lions and tigers, had an unusually high concentration of conjugated histamine[330] which had a dietary origin for parenterally administered histamine produces an increase in free histamine but has little effect on the level of conjugated histamine in the urine[330]

Renal Relations

The part played by the kidneys in the synthesis and/or metabolism of serotonin and histamine in different mammals is obscure Apparently, the kidney of most mammals contains very little or no serotonin, but it has been shown that serotonin appeared in the kidneys of normal rabbits, where it is not normally found[1036, 1040] after the administration of 5 hydroxytryptophan[1036] The results of these experiments appear to indicate that in the kidneys of the rabbit, serotonin is metabolized about as rapidly as it is formed Walton (1956) found that the kidney of the house cat contains an appreciable amount of histaminase but no (at least no detectable) histidine decarboxylase the kidney of normal rabbits contains very little or no histaminase, but has a fairly strong histidine decarboxylase activity[330] and contains no measurable amount of serotonin[1036] These findings indicate that the kidney of rabbits contains no histamine and no serotonin but that it has the enzyme necessary for synthesizing histamine The kidney of hogs is well equipped to synthesize serotonin and inactivate histamine, for it contains appreciable quantities of 5 hydroxytryptophan decarboxylase[1037] and is one of the best sources of histaminase[668, 1040] The kidney of hogs not only contains the enzyme necessary for synthesizing serotonin[103] but contains it in sufficient quantity to be a commercial source of 5 hydroxytryptophan decarboxylase McHenry and Gavin[668] described a method for obtaining from this source a stable powder high in histaminase activity The results should be interesting if the data on the occurrence of the above substances in a wide range of mammalian kidneys were tabulated However Brodie s statement to the effect that serotonin is formed in all tissues of the body following the administration of 5 hydroxytryptophan[114] indicates the ability of all body tissues, as well as the kidney, to form serotonin

There is evidence that other metabolic products of serotonin occur in the urine, some of which apparently have the pharmacological properties of serotonin Ketty calls attention to the work of Bumpus and Page (1955)

weight that has powerful effects even in the very low concentrations found in, or given off by, living tissues This makes detection and estimation of histamine or its releaser by the ordinary methods of chemical analysis, difficult and inaccurate[174] and necessitates assaying 'by use of physiological responses," such as a living muscle[416] Other types of physiological response are obtained by intravenous injection of tropamine blue followed by injection of the sample to be tested[705] If the response is very active the dye will appear in the skin of the animal (preferably guinea pig), wheals are formed from intradermal injection and testing blood,[174] urine or peritoneal fluid following appropriate injections[70] Code[174] warns that the potassium content of the extract should be checked, otherwise final biological tests may indicate the presence of a misleading histamine content Barrum and Gaddum (1935) recommended that all extracts be boiled with acid before being assayed in order to destroy histamine inhibiting or binding substance which they demonstrated is present in extracts of rabbits blood[3, 9] The indirect methods of assay are subject to various errors of technique, the direct methods may result in grave errors as a result of enzymic and physiological action on the test substance within the animals being used

A method based on the application of established statistical procedures of biologically assaying substances for histamine by the use of guinea pig uteri has been developed by Gasler and Matsuba[341] It appears to have several advantages over previous methods although it still has room for improvement This method of assay has an average Standard Error or Estimate about ± 10 per cent but eight different unknowns can be assayed conveniently in a period of sixty minutes using two isolated tissue baths

There are at least three mammalian enzymes capable of destroying histamine but these enzymes are not equally important in all mammals Schayer[889] states that of the three enzymes diamine oxidase (histaminase) is the only important one in rats and histamine metabolizing enzyme II is the most important one in mice diamine oxidase and histamine metabolizing enzyme II are about equally effective in the guinea pig The first enzyme that he considers an acetylating enzyme apparently has only minor importance as a destroyer of histamine but the occurrence of all three enzymes in each of the three species of mammals should have some significance

Histaminase (diamine oxidase[648, 893] but is delimited as to synonomy by others[507, 1134]) destroys histamine Like histamine it is widespread in nature and is abundant in certain normal animal tissues but may vary widely in quantity in the same individual[17] Histaminase is fairly abundant and consistently present in the intestinal mucosa[177, 10] where (in

nephrine and histamine are probably even more significant, especially in controlling the systemic balance of histamine, either as conjugated or as free histamine, or as free or conjugated histamine coupled with diazotized P nitroaniline[1005]

The control of histamine may be by degradation, by combination with some substance (usually protein) by inhibition of formation as by inhibition of histidine decarboxylase, or vasoconstriction, or by physical restraint, as by being "kept away from sensitive tissues by anatomical factors'[39] and, possibly, by adsorption or combination with proteins loosely bound in tissue proteins "by peptide linkage to the carboxyl group '[39] Antigen antibody reaction is omitted since it involves reaction against the antigen rather than against histamine The liberation of bound, linked or otherwise inactive histamine by histamine liberators has been discussed in some detail in a preceding section

The various means by which the formation of histamine from the alpha amino acid histidine may be prevented, include deficiency in source of components defective synthesis deamination and transamination The nature site of action and pathological possibilities apparently indicate that destruction by histidase is the most significant known method of pre venting the formation of histamine by decarboxylation of histidine Histi dase an enzyme which is highly specific for L histidine acts be t at pH 8 to 9 It is found only in the liver[1041] (at least in higher animals)[993] and liberates ammonia and alpha formamidinoglutaric acid from L histidine[993] by 'opening the imadazole ring" [39] Histidase probably inhibits histami nogenesis by competing with histidine decarboxylase for the available histidine which normally may be converted into histamine by decarboxyl ation

Epinephrine antagonizes histamine production by inhibiting histidine decarboxylase from converting L histidine to histamine[648] [1083] Thus as corbic acid and dietary flavones since they potentiate epinephrine[1083] an tagonize histaminogenesis Ascorbic acid is also known to potentiate the effects of the chalcone forms of natural flavones particularly hesperidin methyl chalcone in reducing experimentally induced excessive capillary permeability to normalcy[647] Thus the effects on the action of epinephrine and upon increased capillary permeability appear to indicate that the bi flavonoids[36] especially when potentiated by ascorbic acid are important inhibitors of both the formation of histamine and of its effects on capil laries The idea of hi tamine inhibition by competitive substances which resemble histamine in structure should be mentioned The e analogues "supposedly prevent histamine from reaching those cells or enzymes to which histamine normally becomes anchored or they may replace hista mine in other sites[407] Histamine is an amine of rather low molecular

the increased amount of histaminase immediately destroys "the histamine which may be produced by intermediary metabolism"

Although the results of the foregoing experiments and clinical observations show definite relations between certain hormones and suppression of certain allergic reactions, the results are variable and, for the most part, impractical to quantify

Hormone Histamine Relations

Relatively few studies have been directed toward the relations of hormones to mast cell activities However the results of most of the work in this field indicate that certain endocrines do affect the mast cell population in certain structures where the function of mast cell products is to control vasodilation particularly of the terminal vascular bed [1139] By this means, the amount and content of tissue fluid protein is controlled in localized regions Further, this terminal vascular function appears to be effected chiefly by increasing or diminishing the amount and/or duration of histamine released

Hormones appear to have an important role in the control of histamine but, as Feldberg[301] points out little is known about the influence of hormones on the histamine content of the various tissues It has been suggested that there is a histamine thyroid parathyroid adrenal relationship [888] in which one or more of the endocrine glands may play a more significant part in the control of histamine in the male than in the female rat For example the histamine tolerance may be increased through a greater adrenal antidyne role by the male than by the female hormones [888] It is also recognized by a number of investigators that there is a reciprocal relation between the adrenal glands and the histamine level of the blood so that an excess of one substance stimulates the production of the other until the local or general as the case may be histamine epinephrine balance is again established and maintained [390, 9, 1007] The histamine epinephrine balance is most important in regulating capillary dilation as is shown in the discussion of capillary permeability changes However other factors that may aid in maintaining this balance or in disrupting it are normally present or may be introduced It should be noted at this time that although adrenaline is generally considered to be a pressor agent in certain regions the arterioles and capillaries are dilated by it whereas very small amounts may even have the effect of lowering the blood pressure [9, 964] The more common regions in which epinephrine simultaneously acts as a vasodilator while elsewhere it acts as a vasoconstrictor are the coronary and intestinal vessels and those of skeletal muscle [9] Epinephrine and the derivatives of epinephrine have markedly beneficial effects

terms of "units per gram of wet tissue") it has a value of 3 35 in the intestinal mucosa of the rabbit, about 3 00 in the rat and mouse, and 1 54 in the guinea pig [177] The highest histaminase content recorded by Cohen[177] for any somatic tissue of these 4 common animals is 21 2 in the lung of the rabbit

Inactivation of histaminase is effected by a surprisingly wide array of essential, natural substances ranging from potassium salts to spermine [648] with L histidine listed as being the most specific inhibitor among the amino acids [648] Aminoguanidine almost completely inhibited diamine oxidase in rats[899] and it is a very potent destroyer of the greatly increased histaminase of pregnancy However Roberts (1954) found that subcutaneous injection of a sufficient amount of aminoguanidine to reduce the extremely high level of pregnancy histaminase guanidine to less than 50 per cent, disturbed the normal course of pregnancy with the result that malformation or death of fetuses or of newborn young occurred in many of the rats [504] Histaminase is also inactivated or inhibited by other substances such as hydrocyanic acid hydroxyl amine, pepsin and by as little as 0 0001 M semicarbazide [993] Histaminase activity is destroyed by adrenalectomy or by hypophysectomy [504] It may be restored by the administration of cortisone or cortical extracts, but not by desoxycorticosterone acetate (DOCA) [504]

Histaminase is activated by PO_4[998] and, in the presence of gaseous oxygen, catalyzes the destruction of histamine and most if not all, of the diamines with the formation of hydrogen peroxide [993, 1134] Catalase, which is present in practically every cell apparently is able to destroy moderately excessive amounts of hydrogen peroxide as it invades the cell, but the more massive, free amounts apparently are destroyed by the eosinophils which contain considerable quantities of peroxidase in the form of cytoplasmic peroxidase granules [5] Laidler [77] states that histaminase, in catalyzing the oxidation of histamine and other diamines that contain two or more —NH_2 groups converts the —CH NH_2 group into —CHO A considerable amount of work on the degradation of histamine by acetylation and oxidative deamination has been done in the past few years [899, 1005, 1134]

It is usually assumed that the extremely high serum histaminase activity in pregnancy[507, 1048, 1134] indicates the formation of a correspondingly high serum level of histamine which apparently has not been demonstrated However this tenet may be questioned since the increased histaminase is produced in the placenta[507] and unrefined extracts of placentae act on cadaverine more readily than on histamine [507] Nevertheless, Kapeller Adler[507] supports the probability of the above tenet by hypothesizing that the reason histamine appears to be absent in normal pregnancy is that

renalin versus adrenalectomy are to a certain extent a matter of total dosage and/or duration However the idea that histamine caused the hypersensitivities is partly conjectural and except in certain of the experimental procedures it was quite impossible to eliminate all other factors

Extract of the posterior lobe of the hypophysis cerebri appears to have an antagonistic action on the activity of histamine[1048] whereas hypophysectomy is followed by profound depletion of histaminase[04] Since the administration of cortisone or cortical extracts to hypophysectomized cats and guinea pigs restored the histaminase level to normalcy[504] the obvious interpretation of the effect of hypophysectomy on histaminase is the obliteration of the source of ACTH caused by the failure of the adrenal cortex to produce the necessary cortical substances of which cortisone is perhaps the best known This depletion of histaminase may be prevented or its production restored to normal levels by the administration of cortisone or extracts of the adrenal cortex[04]

Adrenalectomy likewise was followed by profound depletion of stored histaminase[04] and presumably by inhibition of further production of this enzyme Administration of cortisone or of cortical extracts prevented the depletion of histaminase and restored its production to normal levels in the animals having either the adrenals or pituitary removed[04] Halpern[391] and Kahlson[04] believe that adrenalectomy is a fairly definite means of depleting the histamine reserve in the body Ungar[1044] cautions that processes other than those which adrenalectomy may imply are not definitely ruled out for the action of adrenalectomy is devoid of all specificity as is shown by the increased sensitivity of adrenalectomized rats to a large number of agents[390] Ungar's warning could equally well be considered in connection with the effects of hypophysectomy and other major surgical procedures

Experimental results show that the thyroid gland definitely influences the production and course of allergic reactions for thyroidectomy prevented allergic or anaphylactic reaction in experimental animals in which protection was promptly lost after oral or parenteral administration of thyroid substance[1045] Martin and Sackler (1953) found that inhibition of the thyroid gland by the administration of propylthiouracil similarly increased the histamine tolerance of animal[1083] Conversely when plant protein was used to allergize guinea pigs the injection of thyroid increased the severity of the anaphylactic response thyroidectomy subsequent to allergization failed to protect these animals from anaphylactic manifestations[1048] It has also been claimed that hyperthyroidism in man is conducive to asthma but that hypothyroidism and/or hypophyseal insufficiency and certain allergic such as hay fever neurotic edema and urticaria are more commonly associated with asthma[1049]

on histamine and anaphylactic shock by specific action upon the involved structures, rather than a systemic effect[9] However, a sudden release of excessive amounts of epinephrine in response to histamine may cause hypertension[1007]

The relations of hormones to histamine appear to be almost entirely through indirect routes which, under normal conditions, often assume a cyclic balanced pattern Thus, when the balance between histamine and epinephrine is tipped toward excessive amounts of plasma histamine the preponderance of histamine stimulates the production of increased epinephrine[76] which in turn stimulates the anterior pituitary gland to increase adrenocorticotropic hormone (ACTH) production[10] thus causing the adrenal cortex to produce increased cortical hormones[464, 8, 5] Hydrocortisone is very potent in re establishing the balance[8, 5] by causing suppression of the excessive amount of plasma histamine This explanation is supported by Long s (1951) statement that parenteral administration of either epinephrine or histamine causes the anterior hypophysis to release corticotropin[464] Also, excessive plasma histamine would be expected to stimulate the production of epinephrine by causing medullary hyperemia

It is fairly well known that certain hormones have a definite possibly direct, effect on the formation or lysis and/or functions of certain blood cells, but whether or not these cells actually produce histamine in appreciable quantities and finally whether the condition in the tissue being considered is the result of histaminic activity rather than other factors such as inanition, is not definitely shown in all cases Apparently several of the various investigators have assumed that all of the histamine, if it was considered at all was available in the plasma at least, mast cells as a probable source of histamine were often generally ignored For these reasons in this review we shall impress hypersensitivity to serve as an indication of the presence of histamine activity in relation to hormones

It has been reported that the sensitivity of certain individuals to a number of hormones has been proven experimentally by eliminating the factors other than the hormone in question for example the animal proteins and the diluent or vehicle to which the patients or experimental animals might be allergic which, when tested produced Prausnitz Kustner antibodies Thus after applying these criteria to published works Urbach and *Gottlieb*[1049] *are reasonably certain that the following endocrines* actually have caused hypersensitive reactions overdosage of desoxycorticosterone acetate (DCA) epinephrine insulin liver pancreatic or kin extract and thyroxin each caused hypersensitivity Removal of the adrenals, ovaries or pituitary gland enhanced experimental anaphylaxis It appears that the apparently contradictory effects they report such as ad

larly inactivated by the androgens androsterone, epiandrosterone and testosterone, and perhaps less strongly inactivated by cortisone acetate DCA, ethisterone and progesterone He also reported in 1950 that histaminase is activated by natural estrogens but is inhibited by synthetic estrogens Mixed natural estrogens increased the production of histaminase and this activating effect was not altered by adding equimolar amounts of DOCA or progesterone from pregnant mare serum to the mixed estrogens However addition of chorionic gonadotropins or of stilbestrol neutralized or abolished the effects of natural estrogens " Since histaminase, or diamine oxidase destroys histamine any substance which suppresses histaminase should favor production of histamine unless it involves other factors

It is well known that young children afflicted with certain allergies including asthma have a tendency to outgrow ' this affliction during the changes incident to puberty In this connection Brock (1952) conducted follow up studies on 351 asthmatic children and reported spontaneous recovery in 117 and a significant improvement in at least 187 (about 80 per cent) which was attributed to the profound endocrine alterations during puberty [1048]

Presumably, sterilized human females react similarly to castrated male animals [1048] since it has been shown that menstruation may aggravate or even incite certain allergic responses However it should be noted that Eichoff (1939) found in guinea pigs and rabbits that allergization may affect the endocrine glands about as readily as the hormones from these glands can affect allergization [1049] Urbach and Gottlieb[1049] stress the part played by the parasympathetic nerves and those drugs which stimulate these nerves in favoring allergization and in prolonging existing allergic conditions

The number of mast cells and presumably the histamine content has been altered experimentally by injections of estrogens Injection of estrogens into male rats shifted mast cells from connective tissue to the para ard periglandular regions of the mammary gland and produced a new formation of connective tissue which was attributed to the relation of mast cells to fibrillogenesis [3] Estrogen administration may cause several changes in secretion of MPS components in the female reproductive system Mason found that pregnancy decreases mucification in the vaginal epithelium[96] and inhibits its reaction to substances which induce increased mucification of mast cells in the uterus during the puerperium

Mast cells in the uterus may function in relation to effects of estrogens for it has been found that estrogen acts as a polymerizing agent for HA [2] The increase in mast cells in the uterus following administration of estrogens may represent a storage form of highly polymerized HA whereas

SEX HORMONES

It has already been pointed out that most of the effects of hormones on mast cells are indirectly mediated so that in most instances it is very difficult to determine the part actually played by the hormone being studied Nevertheless, there is indubitable evidence indicating hormonal effects on mast cell populations

Estrogen There are a number of preparations of natural and synthetic estrogens which differ somewhat in degree of potency and in effectiveness when administered orally or parenterally Although estrogen is recognized as the hormone controlling estrus in subprimate mammals and menstruation in primates[1007] it is also responsible in conjunction with the anterior hypophysis for the development and/or functional activity of all structures characteristic of the normal adult female Estrogen is employed to inhibit neoplastic growth in testosterone maintained male structures, such as the prostate gland Taubenhause (1953) points out that orchitis, as a complication of mumps can be inhibited by the administration of stilbestrol[10 4]

Estrogen has a number of complex and diverse physiological actions[1007] in addition to producing estrus or the menstrual cycle This hormone has a number of extragenital actions some of which are mediated by increased capillary dilation e g increased proliferation of the buccal especially the gingival), mucous membranes hyperemia of the nasal mucosa and thickening and pigmentation of the skin,[1007] and hypertrophy of the nipples and covering skin in guinea pigs that were treated for 14 days[584] Others pertain to metabolism and/or storage of certain salts water and nitrogen and urinary excretion of citric acid[1007]

Certain animals as well as certain tissues may react differently to chronic administration of estrogen The skin of different strains of mice reacted differently to administered estrogen with regard to thickness and deposition of ground substance which may increase the total thickness of the skin from 10 to 20 per cent in DBA and strain A of C3H mice and 100 per cent or more in Swiss and Hairless strains [7]

Apparently with the exception of certain metabolic relations the chief capacity which enables estrogen to perform its multiple and varied actions is dependent upon its ability to produce and maintain hyperemia in the target structures and restrict permeability of the connective tissue ground substance"[7] until the purpose has been achieved

Existing states of allergy in man are commonly aggravated or enhanced by menstruation the menopause or ovarian dysfunction while any of these conditions most notably menstruation may initiate allergic reactions to foods or various substances from which the individual is allergy free at other times[1048] Kapeller Adler[1007] also found that histaminase was simi

larly inactivated by the androgens androsterone epiandrosterone and testosterone and perhaps less strongly inactivated by cortisone acetate, DCA, ethisterone and progesterone He also reported in 1950 that his taminase is activated by natural estrogens but is inhibited by synthetic estrogens Mixed natural estrogens increased the production of histaminase and this activating effect was not altered by adding equimolar amounts of DOCA or progesterone from pregnant mare serum to the mixed estrogens However, addition of chorionic gonadotropins or of stilbestrol neutralized or abolished the effects of natural estrogens[997] Since histaminase, or diamine oxidase, destroys histamine any substance which suppresses histaminase should favor production of histamine unless it involves other factors

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the increase during the puerperium may also represent a condition similar to that caused by increased polymerization following estrogen administration The increased mucin secretion may also represent a higher polymerization of HA and its combination with sulfuric acid

Preparations of natural estrogens when properly administered stimulate the production of histamine in certain structures in female hamsters[530] if an increase in the number of mast cells may be considered as a criterion of increased histamine production It has been shown that estrogens increased the number of mast cells in the loose interalveolar connective tissue in the mammary glands and in the vascular layer of the uterus of virgin hamsters 42 to 96 days old [30] Although capillary dilation was evident, there was no attempt to correlate this increase in mast cells with the production of histamine In this experiment the hamsters were injected subpannicularly[198] 3 to 29 times so that each animal received a total of 1 4 to 29 0 mg aqueous suspension of estrogens (Lakeside) during varying periods up to 64 days Injections of 0 5 mg (10,000 IU) of aqueous e tro gens or of the vehicle for the suspension of the estrogens, at intervals of more than 48 hours, failed to alter the number of mast cells However, 1 4 to 6 0 mg (22 500 to 60 000, IU) of estrogen given at intervals of 25 hours or less, significantly increased the number of mast cells in the vascular layer of the uteri in the mammary gland, greater omentum and lieno pancreatic mesentery [530, 33]

Repeated, daily administration of e trogens increased the number of mast cells and caused increased formation of AMP and collagen in the mammary glands of female hamsters [530, 33] The administration of estrogens to male rats was followed by a shift in the number of mast cells from the loose connective tissue to an increased number in the interlobular regions of the mammary glands [35]

Testosterone Whether or not androgenic hormones are capable of causing an increase in histamine or histaminocytes (mast cells) has not been conclusively shown However some of the reported activities indicate that testosterone may stimulate ma tocytogenesis and, in this manner cau e an increase in available histamine

More than 40 androgenic hormones have been synthesized but all were derived from natural active testosterone or from its excreted form androsterone [1136] Potent testosterone is obtained from the interstitial cells of Leydig in the te tes of cattle [1007] There are also a number of preparations known by trade names Apparently the term androgen is sometimes used rather loosely to indicate a number of substances having properties of androgenic hormones The laxity in identity of the substance u ed probably stems from the statement that with regard to human testicular androgen the precise nature is unknown [1007]

It has been known since time immemorial that the testes are primarily responsible for the development of masculinity as is demonstrated by early castration. The extragenital activities of testosterone are, in general, similar in the male to those of estrogen in the female. Testosterone has a protein anabolic or nitrogen storing action which is manifested by promotion of growth of the skeleton, muscles and various other structures. When administered to a fasting mammal, testosterone inhibits the breakdown of protein stored within the body and consequently a reduction in the level of protein derived blood sugar.[1007] However, at the same time it may accelerate the depletion of stored fat in compensation and affect the metabolism of electrolytes and water.[1007] For these reasons it is easy to suspect testosterone of playing a part in histamine release and in mastocytogenesis. This suggestion is supported by Rindani's[8 9] finding that testosterone propionate injected into granuloma pouches increased the amount of inflammatory exudate in rats. Taubenhaus,[1010] using turpentine abscesses in rats, found that testosterone as well as estradiol indirectly reduced the formation of granulation tissue by interfering with the supply of growth hormone (STH). He does not state whether or not testosterone affected the exudate.

There is the possibility that a high dosage of testosterone may influence certain aspects of lymphocytic movement. One of 10 adult young male hamsters that received 14 to 30 injections of testosterone (77.5 to 291.5 mg aqueous, Wyeth) during a period of 50 to 146 days showed definite periportal infiltration of lymphocytes and plasmacytes in the liver such as regularly occurs in hamsters and mice[9] that bear malignant tumors.[11] Yun reported that castration of male animals either before or after allergization inhibited anaphylaxis and that the castrated animals lost partial protection after being injected with a testicular suspension.[1048] Apparently the direct effect of the estrogen was to produce hyperemia with increased capillary leakage which was followed by the increase in number of mast cells in the hamsters and presumably also in the male rats.

Progesterone. A direct relationship of progesterone and histamine or of mast cells or other mast cell products to progesterone apparently has not been described. Nevertheless, it is known that administered progesterone increases the permeability of connective tissue ground substance whereas estrogen decreases its permeability as was shown by the use of a dye injected into mice.[57]

Early German writers thought that progesterone was the true "hormone of motherhood" and that estrogen was the "true hormone of womanhood."[1136] However, recent work[173] shows that in the absence of estrogen progesterone is incapable of causing implantation of the fertilized ovum in rats. Cochrane and Meyer[173] found that in ovariectomized rats 45 to

60 hours after mating and injected daily with 4 mg of progesterone implantation did not occur so long as the daily injection of progesterone was continued However, implantation occurred in the majority of these rats within 5 days after 1 μg of estrogen per day was administered in addition to the daily injection of progesterone

Progesterone apparently plays a significant part in water metabolism for the administration of this hormone to hypophysectomized animals causes pronounced polyuria [9] In normal animals it increases the permeability of cardiac cells, and probably other cells and permeable membranes, to potassium and doubtless, other ions [100]

It is well known that pregnancy brings relief in certain types of arthritis, and progesterone has been reported to benefit rheumatoid and, occasionally, osteoarthritis [9] However, neither progesterone nor pregnenolone exhibited any inhibiting effect on the spread of intradermally injected India ink mixed with either saline solution or with hyaluronidase [9]

ACTH and cortisone The effectiveness of hormones in changing mast cells or their number is a debated question Asboe Hansen (1950), Cavallero and Braccini (1951) Fulton and Maynard (1953), and Stuart (1951) are some who believe that the administration of ACTH or cortisone decreases the mast cell count Contrarily, Devitt and co workers (1953, 1954), and Schock and Glick (1953) were unable to observe any significant change in mast cells following this treatment [995] Smith and Lewis (1954) observed no effect on mast cells following adrenalectomy, hypophysectomy or the administration of these hormones [995] Padawer[763] suggests that the changes following adrenalectomy are caused by the operation rather than by depriving the animal of its adrenal glands He, however, found that hypophysectomy was followed by an increase in size of the mast cells and that administration of pituitary growth hormone or of an extract of the gland restored normal size to the mast cells [995] Asboe Hansen and Iversen (1951) report an increase in mast cells of certain regions following administration of thyrotropin to guinea pigs [99]

Adrenalectomy depletes mast cells in the peritoneal fluid as shown by an experiment in which 140 intact mice had 549 mast cells per ml of peritoneal fluid whereas 142 adrenalectomized mice had only 255 per ml of peritoneal fluid, but cortisone produced no change in number of mast cells in the peritoneal fluid within 24 hours [970]

ACTH has at least 19 biological actions assigned to it [60] but interest at this time centers about the relations of ACTH to histamine Since this hormone 'exerts no metabolic effects on adrenalectomized animals,[60] most of the functions attributed to the pituitary gland as regards mast cell products, apparently are mediated through effects of its secretion on the adrenal glands *Thus the relations of ACTH to histamine is that*

ACTH stimulated the adrenal cortex to secrete hormones, especially hydrocortisone[8]. ACTH administered parenterally apparently acts directly upon the adrenal cortex to stimulate the production of cortical substance as indicated by its actions in hypophysectomized animals[94].

Ragan[8, 5] points out that ACTH, cortisone and hydrocortisone all produce a common result in intact animals because apparently both ACTH and cortisone produce their ultimate effects through the medium of hydrocortisone. ACTH, a product of the adenohypophysis, stimulates the *adrenal cortex to produce hydrocortisone or a very similar substance* which in turn produces the end effects usually attributed to administration of ACTH. Likewise, cortisone is converted to hydrocortisone which is the active state of this hormone. Thus, it appears that the end results obtained by administering any of the three hormones are effected through the direct action of hydrocortisone or Kendall's compound F[8].

There are such strong interrelations between the pituitary gland and the adrenal glands that the regularity with which a given dose of ACTH or 11 17 oxy adrenal steroids produces a significant fall in the number of circulating eosinophils in patients having unimpaired adrenal function, enables the clinician to employ this method in appraising the adequacy of dosage and as a simple clinical test of adrenal and pituitary reserve[871]. Under these conditions within 4 hours after intramuscular injection of 25 mg of ACTH, the eosinophil level in peripheral blood is reduced 50 to 80 per cent in man. Failure to obtain this result usually indicates adrenocortical insufficiency[871]. Subcutaneous injection of 0.3 mg of epinephrine as used in the Epinephrine Test will produce a fall of 50 per cent or more in the eosinophil level if the pituitary adrenal reserve is normal and the patient is free from allergy[8, 9, 871].

A major surgical operation on a patient having normal adrenal glands is commonly followed within 24 to 48 hours by almost complete disappearance of circulating eosinophils and usually with a return to the normal level before the 4th day after the operation[871]. Whether the eosinophil leukocyte does or does not carry histamine the above relations of hormones to blood eosinophil levels offer a fairly convenient method of indirectly evaluating the serum histamine level.

Administration *of cortisone to patients or animals suffering from many* of the allergic manifestations incident to excessive amounts of histamine including practically every form of irritation commonly produces tonus of the dilated arterioles and capillaries followed by absorption of the excessive extravascular fluid protein and other substances which have leaked out of the highly permeable precapillaries and capillaries into the tissues. The edematous conditions caused by the administration of a wide variety of substances such as eggwhite[1096], ovomucoid[63], polysaccharides

(including starch, agar and insulin)[867] and antisera[10 6] are, perhaps, somewhat misleading as to their allergic implications It is necessary, therefore, to remember that most if not all, of the edema producing agents are histamine liberators Part of the favorable action of cortisone under these conditions is probably due to the reduction in mast cells,[1 0] with a consequent reduction in histamine liberation Cavallero[1 0] found that cortisone reduced the number of mast cells in organs where they are commonly found in normal rats, as well as in infected animals showing pronounced inflammatory reaction

Histamine and cortisone, or hydrocortisone[8] are antagonistic in at least one important respect histamine dilates,[78 10 8 1087] whereas cortisone tones or constricts arterioles and capillaries[8 3 0 68 683 978 1096]

Freyberg[3 0] neatly summarizes the visible antihistaminic effects of cortisone by stating that it "decreased the magnitude of the whole complex of inflammatory phenomena vasodilation, hyperemia, capillary permeability cellular exudation, leukocyte infiltration phagocytosis and fibroblastic proliferation "

The variable, sometimes inconsistent reports on the action of cortisone on mast cells appear to be contingent upon dosage interval between administrations and/or duration of the course of treatment, as has been shown to be true of its action on lymphoid tissue lymphocytes and plasmacytes in hamsters[06] Also the variable results may be due to some intermediate mechanism or other factors Some investigators report that cortisone has a definitely lytic effect on mast cells others indicate that its effects may range from highly variable to negative[9 0] However most investigators agree that adrenalectomy significantly decreases mast cell[9 0]

Cortisone markedly reduced the rate of resynthesis of cellular histamine in animals previously depleted of histamine by treatment with dextran, Tween or certain other histamine liberators[391 878] By this procedure Halpern[391] has shown that in rats cortisone more than doubles the period between the cessation of treatment with the histamine releaser and the ' restoration of the skin reactivity to dextran This period of latency may be partly explained by Ungar s (1953) reference to Goth and co worker s tenet that cortisone opposes the synthesis of histamine[390] This action probably results from cortisone s interfering with the decarboxylation of histidine by histidine decarboxylase[41] This process is considered to be the probable biological source of histamine[41] Schayer[900] *supports this* idea by tating In my opinion all the histamine bound in the organi m arises from histidine West and Todd[10 4] add that the histamine produced by bacteria in the inte tine is also derived by decarboxylation of histidine Tabor[100] discounts Nash s (19o2) suggestion that *Bacillus coli* can synthe ize histamine from carnosine Further support of the idea that

cortisone inhibits the activity of histidine decarboxylase is supplied by Halpern,[391] who states that the biogenesis of histamine is inhibited by cortisone as was indicated by Schayer and co workers who demonstrated a reduction in the incorporation of C^{14} labeled histidine in the synthesis of histamine in the skin of cortisone treated rats Rose's[5 8] observation that neither cortisone nor ACTH administration affected 'the common test nor the histamine wheal,' both of which may be suppressed by use of antihistamines supports *Halpern's*[391] view that these two hormones act upon the mechanism of biogenesis of histamine in which the vulnerable link appears to be histidine decarboxylase [41]

The idea that cortisone inhibits the biogenesis of histamine[391] appears to be the most nearly acceptable explanation of the relation of cortisone to the inhibition of histamine activity that we have encountered to date This explanation is all the more attractive since investigators are in fair agreement that histamine is derived by decarboxylation of histidine However, this idea does not militate against the theory that cortisone may suppress some hypothetical mechanism which normally has a deterrent effect on the activity of histaminase nor against the tenet that cortisone inhibits extravasation and stasis of tissue fluid

Since adrenalectomy or hypophysectomy destroyed 70 to 80 per cent of the extractable histaminase in cats [504] the administration of either cortisone or ACTH would be expected to stimulate the production of histaminase and the destruction of histidine decarboxylase Thus by increase in activity of two histamine destroying enzymes and contraction of the dilated capillaries the action of administered cortisone or ACTH would form the nucleus for an explanation of the speedy relief in many cases of collagen disease following injection of cortisone However since cortisone and ACTH destroy eosinophils the formation of excessive amounts of H_2O would be expected to pose a possible problem that results from the destruction of catalase in the cells by excess H O unless these enzymes or other agents were capable of destroying the excess $H O_2$

A number of authors have reported that cortisone in normal individuals does not significantly affect the action of histamine or interfere with the action of histamine liberators or of the antigen antibody reaction [391] Likewise the addition of cortisone has no effect on histamine *in vitro*[391] unless the explanted tissue is sufficiently organized to be able to synthesize its own histamine This conclusion was deduced from the results of rat skin culture by Schayer and colleagues who found much less C^{14} histamine in rat skin treated with both C^{14} labeled histidine and cortisone than in the control cultures which were not treated with cortisone They also found that cortisone similarly inhibited incorporation of C^{14} labeled histidine in the synthesis of histamine in the skin of intact rats [391] These results

support the tenet that cortisone inhibits biogenesis of histamine Results of these experiments apparently explain, in part, the basis for the vast number of recorded instances in which the administration of cortisone has been definitely effective in the treatment of a wide range of clinical and experimental allergic manifestations and inflammatory reactions which apparently were conditioned by the liberation of histamine

Another approach is that employed by Goth and co workers (1952) who showed that reaccumulation of histamine in the tissues of guinea pigs depleted of histamine by administration of Tween was prevented by cortisone[878]

The often dramatic effect of cortisone on allergic reactions, including arthritis, may be explained in part as follows (1) It causes normal tonus, if not actual constriction of the dilated arterioles and capillaries[8 136 464 681 711 991 1090] which is accompanied by drainage and consequent speedy fall in pressure exerted by the extravascular fluid (2) It inactivates the synthesis of histamine[390] by suppressing the activity of histidine decarboxylase[1084] and by indirectly stimulating histaminase activity The immediate effects of cortisone are followed by a number of significant although too often reversible, cellular and fluid changes some of which as well as adrenal relations, have been discussed in preceding paragraphs

Desoxycorticosterones The various compounds and proprietary preparations of desoxycorticosterone, *e g* desoxycorticosterone acetate (DCA), desoxycorticosterone (DO) desoxycorticosterone (DOC) (DOCA) desoxycorticosterone diethylacetate desoxycortisone Desoxycortone and other designations) commonly aggravate, rather than antagonize, the symptoms produced by histamine However some act much more slowly than others Some investigators believe that certain of these steroids have a cortisone like, anti inflammatory antiphlogistic[9 9 3] or capillary permeability suppressing action instead of the pro inflammatory phlogistic,[9- 9 3] or capillary permeability increasing action which is characteristic of DOC[390 858 9 9 3 100] Thus, it is generally believed that characteristically the corticosterones increase the permeability of cell membranes (particularly of cardiac muscle cells) but probably of all permeable membranes to potassium and certain other ions and substances[100] The effects of DOC on mastocytogenesis and histamine production are interpreted to a considerable extent in terms of the efficiency of these steroids in producing increased capillary permeability and protein leakage with partial stasis In short the ability of these substances to produce conditions favorable for infiltration of mast cells plasmacytes and other cells common to certain phlogistic sites has been conceded by most writers

DOC is usually considered to have an opposite effect from cortisone on histamine depleted skin of the rat it stimulates resynthesis of histamine

and incidentally, it also promotes the process of inflammation [390] In these respects its effects are more like histamine than cortisone

DCA, or DOCA Acetate (a proprietary brand) would be expected to have activities similar to those of DOC Halpern[391] reports that treatment with DOCA Acetate restored the sensitivity of the skin of rats in which the histamine had been completely destroyed by five injections of dextran in 24 hours which was half the time required by control rats to restore skin sensitivity after the last injection of dextran The skin of these dextran depleted rats when treated with cortisone remained almost entirely unresponsive to dextran at least 96 hours after the last injection of cortisone The statement of Kahlson [94] that DOCA Acetate does not restore histaminase may be taken to indicate that DOCA Acetate favors histamine formation by inhibiting histaminase activity

Irrespective of its mode of operation DOCA Acetate may favor if it does not actually produce arthritic lesions of a rheumatic type This may be interpreted as an indication that this substance may play a part in mast cell and histamine production at the affected atriculations DCA is not only ineffective in the treatment but tends to aggravate the arthritic lesions in rheumatoid arthritis [9] Rats that received a chronic overdosage of DCA often developed acute mono or polyarthritis in the metacarpal or metatarsal articulations during the 2nd or 3rd week of treatment Unilateral nephrectomy apparently had no effect on this DCA produced arthritis although it does affect many of the other conditions resulting from DCA overdosage [8] Patients under treatment for Addison s disease with DCA in high dosage may show acute rheumatic types of changes in certain articulations [9]

Desoxycorticosterone diethyl acetate is reported to have the opposite effect of DOCA Acetate It decreases the flow of inflammatory exudate whereas DCA increases it [8 8]

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to the severity of the deficiency The capillary bed in vitamin C deficient guinea pigs responded to experimental trauma by the formation of petechiae much more readily than in guinea pigs that received normal amounts of ascorbic acid [593]

Zweifach[1138 1139] found that merely stroking the overlying peritoneum with a microdissecting needle caused increased capillary dilation and permeability in the exteriorized mesentery of the mouse It has been found that similar effects are produced by lightly applying a camel's hair brush [1 6]

The relations of mast cell products to changes in the permeability of capillaries apparently involve substances and their effects which are not fully understood A substance indistinguishable from 5 hydroxytryptamine by its chemical and biological properties has been obtained from peritoneal mast cells of the rat [65 894] It was later shown to have been formed by mast cells from 5 hydroxytryptophan [576] This substance 5 hydroxytryptamine or serotonin, was found to be present in the skin and subcutaneous connective tissue of rats in amounts proportional to the mast cell population of these tissues [894]

Since it has been generally conceded that 5 hydroxytryptamine (serotonin 5 hydroxy 3 (β aminoethyl)indole) is a "vasoconstrictor found in sera of mammals "[684] it is difficult to envisage this hormone as a substance which strongly increases capillary permeability Nevertheless results of the work of Benditt and colleagues[6 576 894] certainly indicate that this is the case Rowley and Benditt[884] state that the combined evidence that has been obtained makes it very likely that the edema producing agent released by mast cell damaging substances is 5 hydroxytryptamine" Thus, it appears from their work that the effects of serotonin or peripheral especially dermal capillary permeability are similar to, or perhaps even identical with those commonly attributed to histamine

If one disregards for the moment its synthesizing and storing functions the part played by the mast cell in initiating and sustaining hyperemia and increased capillary permeability by releasing histamine heparin, probably serotonin and other substances is the mast cell s most significant contribution to the physiological and metabolic processes The work of several investigators[7 1 8 6] strongly indicates that mast cells are a very important if not by far the most important source of the histamine that is released in the skin Other investigators have shown that there is a direct correlation between the number of mast cells and the amount of histamine in the skin [670 7 1 8 4 855 1085] In one laboratory it was shown that the skin from the hands and feet of untreated rats contained 61 0 μg/g of histamine but in rats pretreated with compound 48/80 which is nearly specific for mast cells skin from the feet contained only 8 6 μg/g [1] Skin from the dorsum of the normal rats contained 27 9 μg/g and in those pretreated

6

Effect of Mast Cell Products on Capillary Permeability

The physiological implications of changes in capillary permeability are profoundly significant under normal conditions as well as in "disease states"[1130] or in certain, well defined, pathological conditions Proper capillary function is so very important that Szent Gyorgyi[1003] looks upon it "as a mechanism which, in its precision greatly surpasses the finest Swiss watch" He regards capillary fragility as an indication "that this mechanism is out of order" The term "capillary fragility" as used by many writers includes all stages of increased capillary permeability ' but the term "increased capillary fragility should be limited to "chemical lesions in the capillary wall specifically in intercellular substance,'[961] or to other drastic conditions, incuding rupture of the collecting venule of the capillary bed[593] This idea is recapitulated in the statement that an intact capillary system indicates a solvent body[9?]

The work of Kramer and Kramer (1953) indicates that multiple factors are involved in maintaining an intact capillary system[9] for they have shown that hormones of the pituitary adrenal system play a significant part in the control of capillary resistance[1095] Other investigators show that fasting profoundly increases capillary resistance and that realimentation with an isocaloric protein diet rapidly causes the capillaries to return to the state of normal permeability[109] Disturbance in the ionic balance,[593] *in enzymic activity,*[18 19 0] *and in short the activity* of almost any disturbing agent results in change in capillary permeability

Altering the calcium potassium ratios increasing the amount of potassium, or reducing the amount or omitting calcium in perfusion experiments caused swelling of the intercellular cement substance and a great increase in permeability of the capillaries in the living mammal Substituting magnesium for calcium ions also caused the extracellular cement to transform into a jelly like erythrocyte permeable material excessive amounts of calcium hardened the cement and decreased its permeability[1140]

Ascorbic acid deficiency is conducive to capillary fragility in proportion

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with compound 48/80, the dorsal skin contained only 5.4 µg/g.[71] Skin from both regions was rich in mast cells, but that from the back contained only about 75 per cent as many mast cells as that from the hands and feet.[71] However, there are reasons for believing that mast cells also increase capillary permeability by secreting a substance which has a solvent effect on the intercellular cement material of the endothelium and on the adsorbed protein film covering the endothelial surface.[666] If this point can be established, and if the conclusions of Lewis (1927) that markedly increased capillary permeability is dependent upon certain chemicophysical changes in the capillary wall[88] are valid, the significance of mast cells in normal and greatly increased capillary permeability will be very much enhanced.

Mast cells function in the formation of hyaluronic acid (HA) in pericapillary connective tissue ground substance.[18, 20, 4, 150, 6, 966] HA activity opposes the spread of fluid in the tissues[18, 19, 57, 906, 1139] unless it is altered by some substance such as pituitary gonadotropic hormones, possibly estrogens[966] or hyaluronidase.[19, 21, 57, 860] Since it is indicated that hyaluronidase is produced in the connective tissue in anaphylaxis,[970] there is good reason to suspect that this enzyme is produced whenever an excess of histamine is released. Duran Reynals's (1947), suggestion that hyaluronidase regulates capillary permeability[5] apparently supports this view. He also points out that in addition to hyaluronidase, other chemical regulators such as hypophyseal gonadotropic hormones and possibly estrogen play a significant part in increasing the permeability of ground substance.[966] It has been shown by various investigators that several of the bioflavenoids including hesperidin methyl chalone possess effective antihyaluronidase activity and that ascorbic acid potentiates bioflavenoid activity.[860] Demonstration that oral administration of hesperidin methyl chalone reduces the spreading reaction of intracutaneously injected hyaluronidase[860] strongly indicates the significance of HA in preventing increased capillary permeability.

The dilated capillaries and edema produced by topical applications of weak solutions of histamine to the base of human finger nails[76] indicated that hyaluronidase or some other spreading substance had altered the nature of the connective tissue ground substance, presumably by changing some of the HA from a gel like to a sol like state.[57] Indeed because of the resistance to connective tissue permeability afforded by normal HA the formation of a significant edema would, at least as a rule, presuppose degradation of the HA in the connective tissue ground substance.

There is good reason to believe that all cells contain at least a physiological trace of histamine and it has been shown that appreciable quantities

of histamine can readily be released from certain of these cells[86, 17, 66, 301, 391, 59, 896, 1097] However the mast cell appears to be the only cell which contains a maximal amount of stored histamine and at the same time is cytologically and cytochemically adapted to the requirements for general investigation of the relations of the release of endogenous histamine to increased capillary permeability, as the works of several investigators indicate[7, 1, 854, 85, 108]

McGovern[666] suggests that mast cells secrete a "spreading substance" which, by altering "the consistency of the endothelial cement lines" and endothelial surface film, is to a great extent responsible for regulating capillary permeability Apparently, he believes that histamine, heparin and the spreading substance are components of one secretion of mast cells and that they do not exist individually *in vivo* in the rat It is generally conceded that histamine and heparin are stored in mast cells in an inactive, loosely bound form[669, 8, 5] McGovern's[666] suggestion that a spreading substance, histamine and heparin are all three components of 'one secretion of mast cells' is apparently a new approach The presence of 5 hydroxytryptamine, serotonin, a vasopressor substance in mast cells[6, 876] presents a new perspective in determining the functions of the mast cell, for this substance does not seem to harmonize with the effects of histamine heparin, McGovern's spreading substance and acid mucopolysaccharides (AMP) Nevertheless, it is believed that the presence of 5 hydroxytryptamine with heparin and histamine emphasizes the significance of mast cells as participants in the reaction to injury[765]

The view of Manwaring and his colleagues (1923) that all anaphylactic reactions are actually secondary to 'increased specific capillary permeability,' which is the fundamental physiological change evoked in protein sensitization is becoming widely accepted[67] This tenet is supported by the work of Rapaport (1941) and others who found that among allergic children that had this type of capillary fragility about half of them improved following the administration of bioflavone vitamin P, or vitamin K[1049]

Several investigators believe that the abundance of mast cells in a structure is dependent on the amount of connective tissue present[693] I wish to add to this statement our observations showing that there is also a vascular requirement because mast cells do not become numerous in the fat of the so called hibernating gland in the hamster until hyperemia has appeared, and then the increase in mast cells occurs in the region of the fat body where there is conversion of white to brown fat Also it is well established that mast cells have a marked tendency to align themselves along or to "cuff" or "skirt" (Heller's Mastzellenhetten) arterioles and capillaries[693, 8, 3, 1094]

VASOMOTION

The amount of blood supplied to a structure is normally governed by controlling the diameter of the blood vessels which supply that structure, that is, by vasodilation and vasoconstriction. The significant point of vasomotion is that it is the most important primary method by which capillary permeability changes are effected.

Certain agents may effect increased capillary permeability without altering the diameter of the capillaries. These agents usually, by indirect action, alter the physical and/or chemical nature of the endothelium perivascular sheath and/or connective tissue ground substance. However, it appears to be paradoxical to consider the possibility of causing an appreciable alteration in any or all three of these components of the blood tissue barrier without producing changes in the diameter of the involved capillary. Some of the agents or factors usually considered in this category are pH and tonicity changes in the extracellular fluids. Thus, it is pointed out that, in experimental work loss in capillary fluid may be caused by hypertonic solutions of albumin, glucose, sodium chloride or sucrose that shrink and separate the endothelial cells with consequent capillary leakage.[1140] Excessive amounts of sodium fed to hamsters were responsible for capillary changes indicated by numerous petechiae in the capillary bed of the cheek pouch."[93]

The ground substance may be altered by thyroid hormone[1] which may alter its salt water relations and the relations of hyaluronate compounds and connective tissue fibers[1, 1140] and other agents. X irradiation[1140] and the absence or insufficiency of ascorbic acid[860, 1140] hesperidin[647, 860] or other bioflavonoids affect the ground substance and induce increased capillary permeability (or actual fragility) and edema.[593, 647, 860, 1140] Oral administration of hesperidin methyl chalcone has been shown to diminish the spreading reaction of hyaluronidase when injected into guinea pigs.[1140]

Terminal Vascular Structure

The term vasomotion as used in this work is limited to that part of the terminal vascular structure or 'capillary bed'[1138] which has muscular sheets, bands or fibers in the walls.[6, 1138] The true capillaries, the walls of which are free of muscle fibers[1138] are capable of reacting only passively to vasomotion. This may cause variations from the normal tone to various degrees of dilation or constriction of the arteriolar trunk. Consequently the volume of blood which may flow through the capillaries (except possibly, those possessing Rouget cells[6] under conditions of normal blood pressure is controlled by the diameter of the arteriolar trunk. Thus it appears that local volume changes in true capillaries are chiefly the direct result of increased volume (probably with no significant local change in pressure) of blood in the arterial trunk of terminal vascular structures.

The difficulty of identifying the nature of the blood vessel is often complicated by the conditions of the experiment, and by physiological or pathological states. Also, the rather loose way in which investigators sometimes designate capillaries in describing results obtained often causes the reader to wonder whether the involved blood vessel is a true capillary or some other part of the terminal vascular structure. In this review we have followed, as far as it is practical, Zweifach's[1138] work on *in vivo* dissection function and terminology of the terminal vascular structures. Most of his work was done on blood vessels in the mesentery of the mouse. Nevertheless, his structural and functional concepts appear to be in agreement with the accepted pharmacological actions of substances such as histamine and epinephrine that produce known effects on peripheral capillaries.[663, 964]

The terminology and sequence of structures here employed, follow. The terminal vascular structures comprise the arteriole, precapillary, arteriolovenular (a v) bridge' (capillary bridge or "permanently open capillary")[1138] true capillaries, prevenule and venule. The true capillaries arise from the arterioles, precapillaries and a v bridges[1139] and their origins are commonly provided with a muscular or endothelial valve like structure which is opened by dilation of the arteriolar trunk. True capillaries may empty into the venous part of the a v bridge prevenule and/or venule usually they are collapsed during vasoconstriction or some may be collapsed and others contain static blood during the basal level of flow,[56] but they are distended with blood during dilation of the arteriolar trunk of the terminal vascular structure.[1138]

Effects of Arteriolar Changes

Systemic or extensive vasodilation decreased the pressure and rate of flow of the contained blood.[136, 1067] At the same time, however it may greatly increase the permeability of the walls of the precapillaries a v bridges and especially of the true capillaries, and thus permit abnormal amounts of plasma and inorganic ions, inordinate amounts of protein corpuscles and other circulating substances to pass out of the blood stream into the tissues.[136, 108] This increase in capillary permeability is extremely important in normal growth wound healing and maintenance as well as pathological processes. In pathological conditions these changes may present serious problems for under the influence of histamine protein leakage, they may reach 5 to 6 times the normal rate of the affected capillaries.[391]

Since mast cells are rich in stored histamine[7, 1] it is significant that there is a growing tendency for investigators of changes in capillary permeability to attach increasing importance to the release of mast cell

histamine as a provocative factor in increasing vasodilation and thus increasing the permeability of capillaries, especially when confined to a delimited area or region

The consideration of vasomotion *for the purpose of this review* is limited chiefly to the production and control of "active hyperemia[141] with special emphasis on "capillary hyperemia"[141] Local capillary hyperemia results chiefly from the action of an agent that causes relaxation of the muscle fibers in the wall of the arteriolar trunk of the terminal vascular structures particularly in the arteriolar and precapillary regions and, probably, in the a v bridges This is thought to result in increased dilation and a correspondingly increased volume of blood in the arteriolar trunk, which cause consecutive dilation as the increasing blood volume is forced through the relaxed a v bridge and the previously inactive, or even collapsed, true capillaries

Variously Effected Vasomotion

Vasomotion, as delimited in this review, may be produced by any one of a variety of agents which commonly operate by either stimulating or inhibiting the lamina muscularis of the larger blood vessels and/or the terminal vascular structures the muscular bands in the wall of a v bridges, or the muscle fibers which form the valves at the origin of the true capillaries Thus it appears that active vasomotion is dependent upon the contraction of muscle tissue in the blood vessels to produce vasoconstriction and upon the relaxation of this muscle tissue to permit vasodilation There is evidence to support the idea that the vasomotor state of the arterial trunk of the terminal vascular structure not only controls the state of the true capillaries but markedly influences the tone of the aorta and great arteries (including the coronary arteries) and the per minute output of ventricular blood[8] Since the true capillaries of mammals are devoid of muscle tissue,[1138] they are incapable of active constriction but their endothelial walls can exert an appreciable degree of elasticity apparently independently of pericytes or periepithelial cells Ponder[810] *supports this* idea by stating that most observers do not credit endothelial cells with contractility but attribute all active contractility to the 'a v capillaries and metarterioles' which are effective because of the smooth muscle in the walls Thus there is normally a fairly definite correlation between the diameter volume of blood flow and degree of permeability of the wall of the true capillaries and a v bridges and, possibly also involving the precapillaries and arterioles to a certain extent

It has been estimated that in resting skeletal muscles about 90 per cent of the capillaries may be empty and collapsed or in stasis and the re

maining 10 per cent constricted and virtually inactive however, the blood supply to the resting tissue is maintained by a certain alternation of these conditions in the capillaries and during physical activity, blood courses through many of the previously collapsed capillaries [565]

Rouget cells or pericytes, when present may have a function in constricting capillaries [56] However the presence of these cells on mammalian capillaries has not been established [43, 393, 1139] It should be mentioned that the power of active contractility has been ascribed to endothelium *in vitro* by Levi (1923), and *in vivo* by the Clarks and others [5, 810] However some investigators stoutly maintain the inability of endothelium *per se* to contract [5, 810] Nagel (1934) found that capillary endothelium offered little or no resistance to microdissection needles, and that the little resistance present was due to the connective tissue therefore the endothelial cells were very readily deformed by any appreciable force and consequently, endothelial resistance to any force exerted against it is practically negligible since the connective tissue "periepithelium" is chiefly responsible for any resistance to capillary distension [1, 6] However, some investigators believe that the flow of blood through the capillaries can be reduced, or even stopped, by the endothelial cells becoming swollen and distending into the lumen of the capillary [1, 6] We have observed very large cuboidal endothelial cells in certain inflamed human tissues but not in any kind of normal tissue It follows that under normal conditions at least the true capillaries in themselves are essentially inactive and respond primarily to the volume and pressure of blood in the muscular part of the terminal vascular structure which comprises the a v bridge precapillary arteriole and arteriole

There remains the probability that ionic changes and/or other factors including Starling's (1896) principle that increased venous pressure increases capillary transudation [9, 438] may affect capillary permeability independently of the muscular part of the terminal vascular structure Stimulation of the splanchnic nerve was followed by an increase in volume of an intestinal loop while it was enclosed in a plethysmograph Whether the resulting hyperemia was actually caused by stimulation of the vasodilator nerve or merely by relaxation of the intestinal muscle is debatable because it has been shown that any change in intestinal tone is followed by a change in blood flow [1049] Uvnas[1049] supports this latter tenet by pointing out that low concentrations of acetylcholine increase intestinal blood flow, whereas concentrations sufficiently high to cause contraction of the muscle tissue also reduce intestinal blood flow Unfortunately, the fact that the intestinal mesentery is richly supplied with mast cells which store histamine or that the least injury or even disturbance causes the release of histamine apparently was not considered The manipulation of

the intestinal loop incident to enclosing it in the plethysmograph would be expected to release a sufficient amount of histamine to account for the increased blood flow

Recent experiments were made on the fragility of the capillary bed in the cheek pouch by feeding hamsters excessive amounts of sodium, adding 30 per cent of fat and withholding choline or ascorbic acid from the diet of rats or guinea pigs and mechanically or electrically stimulating exposed mesenteric vessels The leakage thus produced was due to defective venules, and demonstrated that the site of the escaped blood during increased capillary fragility was the collecting venule of the capillary bed [93]

Although it is impractical to rule out neuromuscular relations, the contradictory effects obtained with magnesium indicate changes in capillary permeability Deficiency of dietary magnesium caused vasodilation[568] whereas an excess of administered magnesium depressed the blood vascular and respiratory systems in rats [79]

McCollum and co workers[569] found that the addition of only 1 8 parts per million of magnesium to an otherwise adequate diet prevented occurrence of the magnesium deficiency syndrome in rats

The suggestion has been made that some of the various agents which are credited with causing vasomotion act indirectly by causing the release of histamine Whether these agents act by releasing histamine and/or serotonin from mast cells or other cells should not be overlooked, neither should the generally accepted fact that almost any kind of cell injury will provoke the release of histamine be overlooked The relations of histamine and of serotonin to vasomotion are very intimately associated with capillary permeability

HISTAMINERGIC NEURAL ACTION

The action of histamine as a vasodilator is said to parallel that of cholinergic parasympathetic nervous stimuli the action of epinephrine or its derivatives, A 40 and Aludrine[503] as a vasoconstrictor is believed to parallel that of adrenergic sympathetic nervous stimuli Vasodilation is believed to be caused by a neural mechanism[45] and apparently to spread by antidromic or axon reflex[88, 1139] Nevertheless Uvnas[1049] points out that the available experimental evidence indicates that sympathetic vasodilator fibers are exclusively cholinergic and are probably limited in effect to skeletal muscles and possibly to the coronary vessels but that there is very little evidence indicating effectiveness of sympathetic vasodilator nerves to the skin of the dog s ear or to his intestines

The world is indebted to Claude Bernard for the discovery in 1851 of vasoconstrictor, and later of vasodilator innervation in the rabbit[45] However, the mechanism of neural vasodilation as it affects the capillaries

is not as well understood as is vasodilation The vasoconstrictor neurons all belong to the sympathetic nervous system whereas vasodilation is accomplished chiefly by neurons from the parasympathetic but also by fibers from the sympathetic and somatic sensory systems[45] Ample histamine occurs within the sheaths of these nerves, as much as 100 μg/g *histamine dihydrochloride has been found in the sympathetic splenic* postganglionic nerves in the ox[1063] However Von Euler[1063] is not convinced that this histamine serves as a mediator of nerve effects' on smooth *muscle Thus the question of the presence of histaminergic nerves in* mammals is far from being settled

The muscularly walled arterioles are surprisingly well supplied with both *vasodilator and vasoconstrictor efferent autonomic nerve fibers*[387] which appears to indicate that neural control of vasomotion is limited to vessels having smooth muscle in the walls[1140] either forming the tunica *media or occurring as thin bundles or possibly as single cells* Since Zweifach[1138] observed muscle in the wall of arterioles precapillary arterioles and a v bridges but found contractile perivascular elements con*spicuously absent in true capillaries in the mesentery of the mouse* it appears that the smooth muscle in these structures may be subject to autonomic stimulation but that this form of stimulation probably is in*capable of affecting the true capillaries directly* Nevertheless control of the diameter of the precapillary segments of the arteriole affects the diameter of the capillaries by regulating the amount and head pressure *of the blood entering them*

The neuromuscular mechanism suggests an explanation by which non histamine induced hyperemia of dermal areas such as blushing is effected However, the probability of *central antidromic impulses* and of nicotinic action of acetylcholine playing a part in blushing and in certain other instances of vasodilation have been seriously considered[88] Nevertheless Von Euler[1063] states that his and Astrom s experiments that involved stimulation of the ileum of guinea pigs through rather long (7 to 8 cm) pieces of splenic nerve from the ox indicated that the transmission of *neural stimuli is through the release of histamine Several other* investigators believe that a chemical mediator such as histamine is involved in neural stimulation[78] Others suggest that enervation affects capillary sensitivity to histamine *in a manner which is not completely understood*[78] If this is the case in the normal animal it would appear that vasodilation is generally effected by the release of histamine However other writers *indicate that histamine alone or adenylic acid alone could cause capillary* dilation for histamine causes arteriolar dilation in the dog monkey and man adenylic acid like histamine is released by tissue damage and also causes arteriolar dilation[4] *Other writers discourage this idea by stating*

that, in vasodilation, histamine has a direct action on the true capillaries, but that it dilates arterioles "indirectly through the axon reflex."[307] Tonutti[104] suggests that hormones may condition the tissue's reactivity potential to an irritant. Boyd[100] indicates that still others believe that, although histamine dilates capillaries, it contracts arterioles. This last *conclusion is open to discussion, for investigators apparently agree that,* although perfusion of the vessels in excised structures commonly produces arteriolar contraction as opposed to dilation in intact animals,[76] it probably never has an opposite vasomotor effect on capillaries, certainly not on non-muscular, or true capillaries.

Feldberg (1955) suggests that one effect of a large dosage of histamine may be neuromuscular block.[100] He does not state whether or not this neural effect is related directly to increased capillary permeability. However, he believes that the muscular weakness or paralysis of rats and cats, following injection of large doses of either histamine or of histamine liberators such as compound 48/80 may be entirely accounted for by the *injected* or released histamine either producing a neuromuscular block or affecting the muscle directly, with the result that stimulation of the motor nerve becomes ineffective. In 1956 he stated that the muscular weakness and paralysis seen in rats after the administration of a heavy dose of compound 48/80 "is certainly due to neuromuscular block."[100] The results of these experiments in producing muscular weakness by injecting the histamine releasing *compound 48/80 are very significant because they indicate that mast* cells are the chief source of the histamine. Mast cells are fairly numerous in the internal perimysium of skeletal muscle in hamsters and compound 48/80 is considered to be almost, if not quite specific for the release of histamine from mast cells.[0, 721, 855, 857]

Dale (1910, 1911) found that intravenous injection of histamine depressed blood pressure profoundly in monkeys, dogs, cats and *fowl, and that* this result was caused by the primary action of the histamine independently of "the integrity of the sympathetic nervous system."[76]

A novel approach to this general problem was made by Barany and Nordqvist (1949) who found that nerve block, caused by an histaminolytic or typical local anesthetics such as procaine, could be removed by a histamine solution.[648] They also found that when histamine was added to the solution containing a blocking agent such as procaine, the property of the local anesthetic was lost. This antagonism between local anesthetics and histamine suggested to Martin[648] that since "all histaminolytic agents are local anesthetics" there is the probability that by the use of Barany and Nordqvist's technique for nerve block, it should be practical to correlate the action of local anesthetics with their antihistaminic power.

EPINEPHRINE

Vasodilation results from intravenous infusion of epinephrine[964, 1097] as well as from histamine Epinephrine unlike norepinephrine, "within the physiological range' has an over all vasodilator effect and causes increased blood pressure by increasing the output of the heart[1094] The splanchnic and peripheral blood vessels may be powerfully constricted following intravenous injections of epinephrine which acts upon the muscular layer in the vascular wall in proportion to its thickness Consequently, the arteries are more strongly contracted than the terminal arterioles[964] Local injections of epinephrine produce local vasoconstriction intravenous injection of epinephrine into normal animals produces a sharp, though not sustained rise in blood pressure and an increased amplitude in heart beat, in vagotomized dogs the blood pressure may exceed 300 mm of mercury[964]

Staub (1946) attributed the vasodilation that followed intravenous administration of epinephrine to the effects of histamine liberated by the epinephrine since he observed a decidedly increased rise in plasma histamine[1087] Whelan[1087] observed that there was pronounced vasodilation in the forearm which was followed by a sustained increased flow subsequent to the intravenous injection of epinephrine into a human subject He attributed this sustained flow to the appearance in the forearm of ' some non histamine dilator substance from a site of release elsewhere in the body, since he obtained no increase in plasma histamine in the forearm by injecting epinephrine into the brachial artery

The reported variable often contradictory effects of epinephrine on vascular structures is not as easily explained as may be imagined because serotonin histamine acetylcholine and epinephrine each constricted the isolated carotid arteries of swine Epinephrine not only constricted the walls of the carotid arteries but also constricted the vasa vasorum of these arteries However neither epinephrine nor histamine was able to cause constriction of the vasa vasorum of coronary arteries of either man or swine but acetylcholine readily caused them to con trict[9 3]

HISTAMINIC EFFECTS

Notwithstanding the fact that there are many agents and conditions capable of causing vasomotion the most important single point to be discussed at this time pertains to histamine produced hyperemia and the concomitant increase in capillary permeability which may be local or extensive, and which may involve the entire structure or body A disturbing factor in discussing histaminic effects is that it is apparent from the work of Benditt and his colleagues[6, 676, 894] that in an undetermined number of instances effects due to the activity of serotonin or to the combined effects

of this substance and histamine may have been erroneously credited to histamine, or vice versa It has been established that histamine can cause *increased capillary permeability* *Whether it acts directly by dilating the* capillaries or indirectly by constricting the arterioles and venules or acts on all structures of the capillary bed simultaneously has not been definitely settled Rocha e Silva[863] carries the idea much further by stating that histamine constricts the small arterioles and venules, but dilates the capillaries and increases their permeability He adds that histamine is the only substance which has been shown to be capable of thus effecting dilation and of increasing permeability of blood capillaries He supports this idea by pointing out that irritating toxic, inflammatory and other agents which induce increased capillary activity ' do so chiefly by causing the relea e of histamine Irrespective of what time may prove concerning the relative and actual values of serotonin and histamine as edema producing agents, the earliest published work on this function of serotonin appears to be that of Rocha e Silva[863] and Benditt and his co workers[6 884]

It has been suggested that changes in capillary permeability may be effected without producing a readily detectable change in the capillary wall A possible example of this effect may be attributed to changes in the protein film covering the endothelial surface[1138] and in the consistency of the endothelial cement substance and surface film brought about by the 'spreading substance secreted by mast cells[666] or hyaluronidase[19 7] Lewis' (1927) tenet that chemical and physical changes in the consistency of the capillary wall are essential for increased capillary permeability[88] strengthens this point of view

Histamine is a powerful vasodilator[1037] however, there is a considerable amount of evidence indicating that it (probably aided by other agents including other mast cell products) also induces physicochemical changes in the components of the capillary wall[5 666 1140] These changes acting in conjunction with the increased volume of blood and possibly various other factors[18 19 1 85 88] are capable of increasing capillary permeability

Histamine has been found to stimulate endothelial cells to phagocytize foreign lipoid cholesteremic and other materials It has been shown that this phagocytosis is prevented by antihistamines[5] Heinlein (1935) believes that histamine causes endothelial cells to swell and subsequently palisade or even to become detached

Various investigators believe that x irradiation increases capillary permeability but they are not agreed on the mode of its operation in causing the increased permeability Although there is no injury to the endothelium Dahl (1937) thinks that irradiation injures cells in the extravascular tissues and cau es them to liberate the histamine which in turn increases capillary permeability[5] Elf kind (1940) maintains that x irradiation

causes endothelial nuclei to swell, round up and lose their nucleoli[5] Similar conditions often prevail in instances of pathologically increased capillary permeability That x irradiation produces capillary dilation in the skin, if filters are not used was known to the early radiologists who employed the skin erythema dose (SED) as a base for reckoning dosage

These observations and conclusions indicate that released histamine may cause capillary injury and, concomitantly increase permeability The well known tendency of mast cells to aggregate along small blood vessels and capillaries *indicates that mast cells are a likely source of this histamine* However, it is fairly evident that the mature mast cells (Type II) in the neighboring connective tissue are the source of most of this histamine rather than those adjacent blood vessels which are chiefly immature (Type I) cells[8]

Death or injury of any cell, or even mild disturbance of some cells, probably causes or evokes the release of histamine Nevertheless, it is only comparatively recently that many of the more obscure instances in which histamine is released have been associated with increased capillary dilation and permeability For instance, erythema or redness of the skin has been recognized as a hyperemia for many years but the more modern clinicians and investigators usually attribute this condition to such causes as irritation or photochemical reactions Despite the weight of evidence indicating that histamine is an instigator of vasodilation and increased capillary permeability this idea is modified or rejected in part or entirely by certain investigators

Analysis of the over all problem of the relation of histamine to hyperemia and increased capillary permeability is extremely complicated under normal physiological conditions The analysis is further complicated by experimental procedures by disease states[1130] including such conditions as factitial urticaria[88] or dermographia and by pathological conditions In addition there remains the probability that multiple factors are involved

DIVERSE RELATIONS OF HISTAMINE

Histamine causes marked contraction of intestinal and uterine smooth muscle of the guinea pig[76] An isolated piece of guinea pig s uterus reacted by contracting when placed in 1 250 000 000 histamine in Ringer s solution Also histamine caused the contraction of excised pieces of uterus of the dog and rabbit, but had highly variable effects on the uterus of mice it usually inhibited contraction of excised pieces of rat uteri[6]

Histamine is a potent vasodilator of intact blood vessels[1087] but as described below perfusion with it usually causes the blood vessels in excised structures to constrict However it is doubtful if histamine plays an

important part in vasodilation which follows exposure to certain conditions, such as, immersion of the fingers or hand in cold water, temporary arrest of delimited circulation exercise and epinephrine infusion of the human antebrachium [1037] Nevertheless Code[17] holds that 'stimulation of gastric secretion *is* a function of histamine," and the work of others[76 371] supports this view A sustained increase in glandular secretion is effected through hyperemia, and histamine is known to be a potent agent in causing va odila tion and increased capillary permeability [175] The ability of this amine to cause increased volume and acidity of gastric juice should be attributed primarily to histamine produced hyperemia Subcutaneous injection of compound 48/80 (the powerful histamine releaser) into dogs or its intra venous injection into cats is followed consistently ' by the secretion of large quantities of highly acid gastric juice " This report supports the contention that increasing the secretion of acid gastric juice, by "increasing the blood supply of the gastric glands," is a function of histamine [175]

Several investigators have made a point of the distribution of mast cells in the wall, especially in the adventitia, of the aorta and great veins[99] and of their characteristic of aggregating around (cuffing'), or aligning them selves along, small vessels and capillaries [438 693 8 8 4] Mast cells often form a perivascular sheath of immature, non metachromatic mast cells (Type I of Riley)[8] - around muscularly walled arterioles and true capil laries [8 8 4] Numerous mature metachromatically staining mast cells (Type II of Riley)[85] are scattered throughout the loose connective tissue farther away from the blood vessels These two distributions of mast cells appear to assure an adequate supply of histamine for unlimited, directly histamine produced vasodilation or for transfer of stimuli from vasodilator nerves [45]

If mast cells are an important source of histamine one is justified in as suming that *these* mast cells are an important source of the histamine which undoubtedly plays a significant part in vasodilation wherever they occur in connection with blood vessels and that this significance is related to the density of the mast cell population in the adventitia and neighboring connective tissue

The effects of histamine and its antagonists on the normal terminal vascular structures and their functions bear a fairly definite relation to the architecture of the small blood vessels and may involve also the lymphatics The tone and response of muscle tissue in the wall of arterioles precapil laries and a v bridges and the dilation and contraction of the true capil laries are essential to maintenance of the blood supply demanded by the condition of the tissues involved [1138] These physiological states in normal animals vary from the basal, or resting level of tissue activity in which the contracted arterioles, precapillaries and a v bridges carry practically all

the arterial blood directly to the prevenules while the true capillaries are virtually empty and may even be collapsed to the hyperemic state in which the arterioles and a v bridges are dilated and the true capillaries may all be greatly distended with blood[1138]

MECHANICS OF CAPILLARY RESPONSE

It is extremely difficult to explain the mechanics of changes in capillary permeability because investigators are not in agreement as to the structure of the capillary wall,[5, 1138, 1140] particularly of the endothelial membrane,[5, 663, 1059, 1138, 1140] or the significance of chemical and physical changes in the luminal adsorbed protein film and/or in the perivascular connective tissue[5, 1, 2, 7, 3, 6, 563, 666, 1139]

Changes in capillary permeability usually result from changes not only in the capillary wall, which probably includes reticular and elastic fibers and collagen,[5] but may also involve all entities that form the structure of the hematoparenchymal barrier This may include changes in the adsorbed protein film that covers the luminal surface of the endothelium,[666, 1138, 1140] in the basement membrane,[5] and in the endothelial cells themselves,[5] and also in the consistency[1138] and porosity[5, 840, 1138, 1139, 1140] of the endothelial intercellular cement substance The pericapillary adnexa includes elastic, fibrous and collagenous connective tissues and part of it is organized to form the ' pericapillary sheath " The amorphous connective tissue ground substance which contains HA[1, 7, 356, 563] and collagen[38, 549] forms probably the most important part of the blood tissue barrier[966] The endothelial membrane serves chiefly as the skeletal framework' of this barrier[1140] This idea was developed further by Duran Reynals (1947) who believes that capillary permeability is regulated by hyaluronidase which is very important in controlling the permeability of the ground substance[356, 563] Chambers and Zweifach (1947) think that the action of hyaluronidase causes capillary fragility[5] Drinker (1927) perfused the web of the foot with extracts of pituitary gland and horse serum[565] He concluded that a hormone is present in frog blood which is capable of restoring and maintaining normal capillary permeability in capillaries previously made permeable to colloids

Hyaluronidase evidently plays a very important although indirect role in controlling capillary permeability by altering the viscosity of the HA in the pericapillary connective tissue and its ground substance The idea that the anti inflammatory effect of adrenocortical hormones is mediated chiefly through the activity of these hormones in increasing capillary resistance and the consequent decrease in capillary permeability[363] sheds some light on the relation of viscosity changes in HA to permeability changes in the capillaries Other work[356] shows that the anti inflammatory action of cer

tain adrenocortical substances affects HA by increasing "the hyaluronidase inhibitor levels" in the blood serum This increase in hyaluronidase inhibitor adds to complications of transportation but does not militate against the concept of cortical control of hyaluronidase Five of 36 adrenocortical hormones (1 mg/100 g body weight/day for 3 days), including cortisone acetate, increased capillary resistance as shown by the abdominal skin test in rats previously accustomed to handling 50 to 60 cm Hg over that of control animals [63] In the light of the results that were obtained by other investigators [356] this increase in capillary resistance should be attributed chiefly to the potency of cortisone and certain other adrenocortical hormones in suppressing the activity of hyaluronidase and the consequent increase in viscosity of the HA

Sommers[966] stresses the significance of normally intact basement membranes and connective tissue ground substance He points out that localized dissolution of basement membranes sometimes precedes parenchymal atrophy of aging organs Although his emphasis is upon organs it is evident that capillary permeability changes are the motivating factors

Kisch (1955) attaches great significance to the wall and adnexa of the capillary in regard to the complexity of its structure and relation to permeability changes in capillaries as revealed by the use of the electron microscope [1140] He regularly observed an unidentified substance between the capillary wall and its sheaths He makes various comments on the complexity of capillary structure and stresses the significance of changes that occur in the capillary wall under various conditions especially as seen in ascorbic acid deficiency

An important point in the mechanics of capillary permeability pertains to the physical state of the capillary wall In true capillaries of the mouse's mesentery the capillary wall is composed of elastic endothelium that 're mains the sole contractile element [1138] and in the distended state exhibits stigmata or perforations [840, 1138] Thus since the wall of the true capillary is elastic and porous increased blood volume in the arteriolar trunk and the related increase in capillary dilation may increase leakage of protein molecules at a rate much higher than would be suggested by the actual increase in diameter of the capillary Vasomotion of the true capillaries in normal and inflammatory conditions could be effected without any direct influence being applied to the true capillaries other than the elasticity of the endothelial walls which would function only in producing a certain degree of tonus in the capillary Any vasomotion affecting the a v bridge precapillary arteriolar region of the artery would be immediately transferred as a blood pressure change in the true capillaries Thus inhibition of the muscular region of a terminal arteriolar structure would cause dilation and if sustained an increase in capillary blood pressure which would favor exuda

tion These relations could account for Menkin s[68] finding that the avail able data indicate that capillary dilation in inflammation or simple hyper emia is accompanied by increased capillary pressure Landis (1934) also thought that arteriolar dilation is responsible for "the rise in capillary pressure in hyperemic conditions ' [68]

Various writers have pointed out that increased capillary permeability is commonly associated with dilation in a large number of capillaries and that capillary permeability can be increased until any colloid will pass through the wall However, some dilation may occur without any per ceptible increase in permeability whereas a high degree of permeability may be attained without appreciable dilation of the capillaries [565]

PERMEABILITY CHANGES

Changes produced experimentally in capillary permeability may be ex pected *primarily to involve the entire terminal arteriolar structure both* the muscular part and the muscle free part which compri es the true capil- laries

Hyperemia is essential, but the work of Lewis (1927) indicates that, in addition to increased blood flow it is necessary that a physicochemical ('qualitative) change takes place in the capillary wall before its per meability, especially to proteins can be markedly increa ed [88] He applied suction (90 mm Hg negative pressure) to sensitized human skin (red line) and increased capillary pressure by interrupting venous return This method failed to promote whealing and 50 mm Hg positive pressure on the ensitized skin did not prevent the formation of wheals

Capillaries are extremely sensitive to environmental changes as well as to irritation or actual injury Their sensitivity is indicated by the altered permeability of the walls of disturbed capillaries The amount or extent of disturbance which is necessary to provoke an increase or decrease in capil lary permeability is so small that it does not need to produce a physical change in the involved capillaries Zweifach[1138 1139] found that merely stroking the peritoneum of the mesentery with a microneedle produced a pronounced increase in the permeability of nearby but untouched capil laries in the mesentery of mice Hyman and Chambers showed that capil lary permeability can be decreased readily without any detectable decrease in diameter or other physical change in the capillaries of rabbit [10 9] They attributed this decrease in permeability to a change in the endothelial cells of the capillaries and stated that this condition may be induced by ex tremely weak solutions of adrenocortical hormone Contrarily Mc Govern s[666] spreading substance which increases capillary permeability, and which he suggests is secreted by mast cells would be expected to in crease endothelial permeability without causing perceptible change in cap

illary diameter by altering the consistency of the intercellular cement and endothelial covering film, provided its actions were not augmented by released histamine

Normal Capillaries

These observations suggest that it would be advisable to consider some of the points concerning permeability changes of normal capillaries Under normal conditions maintenance permeability, which may be considered primarily as supplying the normal amount of transudate, appears to be a function of the a-v bridge, with possible contributions from the arteriole and precapillary, the true capillaries are primarily collapsed or otherwise inactive most of the time[56, 1135, 1139] Under conditions of normal maintenance, a higher level of permeability of controlled duration is induced probably for the purpose of permitting passage of proteins, other substances and added amounts of serum Conceivably this cyclic process may be initiated by an infinitesimal amount of mast cell secretion, which may be released by dialysis rather than by cytolysis of the involved mast cells or, possibly, by neural stimuli

Renkin[840] points out that the normal endothelial cell, like many other kinds of normal cells that have diverse origins and functions, has a much higher degree of permeability to lipid soluble molecules than to lipid insoluble molecules He suggests that the lipid insoluble molecules may be largely restricted to passage from the capillaries through the stomata He also states that the freedom with which lipid soluble molecules oxygen and carbon dioxide permeate the capillary wall may be the result of increased permeability of the endothelial cells themselves rather than a change in the intercellular cement or the stigmata This application of differential rate of dialysis through the endothelium is further complicated by changes that may occur in the adsorbed protein membrane covering the endothelium the endothelial membrane itself and/or the pericapillary connective tissue and its ground substance

Landis (1934) has shown that injury to a capillary may increase the amount of escaping fluid as much as 7 times the normal amount[683] This leakage is the result of vasodilation which causes dilation of the capillary pores[663, 840, 1138, 1139] Estimates based on the known size of particles which passed through gave maximum values around 38 Å for the diameter of the pores in normal capillaries and up to 200 Å for the diameter of the pores in dilated capillaries resulting from injury[663]

The presence of capillary stomata is not universally accepted because some investigators believe that capillary walls do not have actual stomata or any type of preformed openings that are designed to permit the passage of materials into the intercapillary areas[387, 56] Others believe that stomata

are only temporarily formed in the cement substance and basement membrane by liquifaction some doubt the presence of a basement membrane in any blood vessel [5, 565]

The ground substance of the connective tissue in which the capillaries are embedded normally has a gel like or slimy viscous consistency [1, 2, 3] When the capillary is stimulated with microneedles the endothelium loses its elasticity, becomes sticky, and the connective tissue ground substance becomes increasingly liquid thus greatly favoring the spread of exudate into the tissues This process occurs in the mesentery of the mouse cat and frog [1139] Other investigators have demonstrated a similar reaction in exteriorized mesentery of the frog [56] rat dog and cat [1139]

The blood vessels of ascorbic acid deficient guinea pigs formed petechiae during experimental trauma much more readily than those in normal animals In at least 90 per cent of the instances the escape of the formed blood elements was through the collecting venules in the capillary field most often through a tricornered tear but in some instances through the increased porosity of the venular wall [593]

Mechanical Irritation

Extremely delimited areas of injury (10 to 15 μ) are known to release histamine and to produce capillary dilation with increased capillary permeability [1138, 1139] Zweifach [1139] showed in a film that gentle irritation produced by tapping the wall of a capillary with a microneedle caused dilation of the capillary with such marked increase in the diameter of the so called stigmata or capillary pores [663] that they consistently trapped erythrocytes In one instance the film shows a capillary with an erythrocyte being extruded through a tiny opening and pinched into two parts Since any injury may be expected to release endogenous histamine this would be the logical explanation of the increased capillary permeability However Zweifach [1139] was unable to obtain any evidence to establish unequivocally the participation of particular substances such as histamine leukotaxine etc in these vascular reactions

Zweifach [1139] by injecting graphite showed that the surface of endothelial cells normally is not sticky He observed no tendency for platelets to adhere to the walls of arterioles or to each other in otherwise normal mice However irritation with microneedles presumably by releasing histamine heparin and possibly McGovern's [666] spreading substance from mast cells initiated vascular dilation and the stickiness with the result that carbon particles as well as platelets and leukocytes adhered to the endothelium Even the slightest injury such as that caused by gently stroking the endothelium with microneedles without touching the capillary produced stickiness within the capillary just proximal to the site of irrita

tion, more severe irritation of the vessel caused stickiness distal to the site of the injury When the immediate vicinity of a terminal arteriole was intensely stimulated mechanically by injury or gentle rubbing with the microneedles for 30 to 40 seconds, blood platelets accumulated on the endothelium and "adhered to one another forming long strings or chains" within the arteriole at the site of irritation [1139]

Exogenous Histamine

Intradermal injection, topical application or other means of exposing tissues to histamine may provoke capillary reaction and, usually, increased permeability of the walls of the involved capillaries Investigators in 1910 and 1911 showed that histamine injected intravenously into anesthetized fowl, cats dogs and monkeys, profoundly reduced blood pressure [76] Dale and co workers (1910, 1918) found that vasoconstriction resulted when excised structures of the cat were perfused with histamine, but if erythrocytes or epinephrine were added to the histamine solution before perfusion, vasodilation was produced in these structures [76] Histamine perfusion of these structures in the intact, control cats also caused vasodilation [76] Florey and Carlton (1926) injected histamine into the saphenous vein of anesthetized cats, and they observed the dilation of mesenteric capillaries and venules and the opening of collapsed mesenteric capillaries [76] They also observed that the removal of a piece of the mesentery was followed by constriction of its venules and, when pituitrin was applied to the omentum that had capillaries fully dilated by the histamine the capillaries definitely constricted Similar effects of histamine were observed by Rich (1921) who found that injection of histamine produced local dilation of small arterioles capillaries and venules in the mesentery of anesthetized cats [76] He also observed "new capillaries ' opening up in the omentum as a result of the injected histamine

Topical application of weak solutions of histamine have been shown to be very effective in causing vasodilation and increased permeability Carrier (1922) demonstrated dilation of the capillaries in normal human subjects with accelerated capillary flow accompanied by edema following applications of histamine that were diluted 1 1000 1 5000 and 1 10 000 to the base of the finger nail [6] Feldberg (1927) and Flatow (1929) applied histamine to the rabbit's ear and observed dilation and opening up of new capillaries [76]

Endogenous Histamine

Rocha e Silva (1953) states than many observers have shown that in sensitized animals antigen injury provokes platelet response in capillaries similar to that following microtrauma and that this reaction apparently

is connected with the liberation of histamine and heparin from hepatic cells in the dog.[1133] Rocha e Silva's connecting the above effect of microtrauma and antigen injury with liberation of histamine appears to be very logical, but does not preclude the possibility that adenylic acid, which is released by tissue injury and causes arteriolar dilation,[4] may play a part. However, with an abundance of mast cells within the adjacent connective tissue and the presence of blood platelets which Dale (1936) believes contain great amounts of histamine in the rabbit but none in the horse,[86] it would hardly be necessary to look to the liver as the source of the active histamine and heparin. Speirs[0] extends and in part supports Rocha e Silva's tenet by pointing out that antigen introduced into sensitized animals, especially when it produces anaphylactic reaction, may provoke the release of several substances including heparin, histamine, hyaluronidase, possibly choline and other active substances, to which many of the observed symptoms may be attributed.

Lewis and colleagues (1927) neatly showed that the triple response and whealing are directly dependent on increased capillary permeability.[88] These investigators found that if, after arresting the circulation by compression, the mechanical stimulus was applied to the skin, whealing failed to develop as long as the circulation was prevented, but as soon as the blood was allowed to flow, the triple response immediately appeared.

Reference has repeatedly been made in this review to various types of experiments which show that injury, irritation or almost any form of disturbance of living tissue will provoke the release of histamine, capillary dilation and increased capillary permeability in laboratory animals and man. Because in many ways endogenous and exogenous histamine produce similar or even identical results, as in so-called serous inflammation and whealing,[89] a doubt is raised concerning the practicability of trying to consider exogenous and endogenous histamine separately. This distinction usually is readily made in experimental work. When it is applied to insect bites and stings, or to contact with poisonous or allergic plants or other agents, it is often difficult to determine whether the offensive histamine was introduced into the tissue or released within the tissues by the introduced substance, *e.g.*, formic acid in bee sting. The demonstration of considerable amounts of histamine in nettles, lamb quarter, tomatoes, spinach (0.5 mg/g in growing seed, up to 1.34 mg/g in flowers of over-wintered plants) and other plants[301] may add to this confusion.

INFLAMMATORY PROCESSES

The universal presence of increased capillary permeability in inflamed tissues and in those undergoing amyloid degeneration is attributed by Lewis (1927) to the presence of histamine and/or its related H substance.[86]

tion, more severe irritation of the vessel caused stickiness distal to the site of the injury. When the immediate vicinity of a terminal arteriole was intensely stimulated mechanically by injury or gentle rubbing with the microneedles for 30 to 40 seconds, blood platelets accumulated on the endothelium and "adhered to one another forming long strings or chains" within the arteriole at the site of irritation.[1139]

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Rocha e Silva (1953) states that many observers have shown that in sensitized animals antigen injury provokes platelet response in capillaries similar to that following microtrauma, and that this reaction apparently

listed them as rubor calor tumor and dolor, a terminology and sequence of events which time has not altered[6, 9, 633, 663] Both *rubor (erythema)* and calor (heat) are due to hyperemia tumor (swelling) and dolor (pain) are the result of increased capillary permeability[6, 9, 633, 663] and resultant pressure[94]

The physiological changes which local circulation undergoes in an inflamed area were recognized and treated by Cohnheim Samuel and Virchow Samuel in 1873 recorded his belief that changes in the walls of the blood vessels give origin to the exudate which forms the fluid in exudative edema[68] However Cohnheim (1866 and later) paved the way for further experimental interpretation of inflammatory processes[68] and edematous relation

Ehrlich[71] briefly summarized the theories on the mode of origin of inflammation from humeral to vascular and phagocytic concept He supports the tenet that inflammation may be regarded as a disturbance in homeostasis provoked at least in part by tissue injury and the release of histamine Lewis (1924 1927) results support Ehrlich s conclusions by indicating that erythema following ultraviolet is caused by the release of histamine within the skin[88] Ellinger (1928 1930) believes that ultraviolet and x irradiations cause an increase in histamine in the skin by killing some cells and by decomposing histidine photochemically[19] Ehrlich (1953) also states that Rössle believes that instead of the vascular reaction being an essential part of inflammation it may be considered as an auxilliary mechanism' which has the function of accelerating removal of the cause of the inflammation[484]

Although Roessle s explanation is quite tenable hyperemia and increased capillary permeability will probably remain the most significant characteristic for the recognition of inflammatory processes Ungar[1045] in his plea for some measure of agreement on the definition of inflammation stresses the idea that inflammation is primarily a vascular response Dorland[39] states that inflammation is characterized histologically by hyperemia stasis changes in the blood and walls of the small vessels and by various exudations '

Mast Cell Relations

A number of pathologists have recorded the presence of mast cells in inflammation and state that they may be especially numerous during chronic inflammation[603] Many investigators have noted that the density of the mast cell population is directly related to the proximity of small blood vessels and that this relation is commonly demonstrated by aggregations of mast cells around small vessels[697, 8, 7] This arrangement may be a form of reciprocity in which the mast cells receive needed substances from

Incidentally, Lewis H substance is now known to be histamine[88] That histamine is capable of increasing capillary permeability is well established and it has been shown that serotonin released from mast cells is very potent as an edema producing agent [70]

There are certain factors which militate against presenting clear cut indubitable evidence to show the extent to which mast cells are a source of the histamine serotonin and/or other substances concerned with hyperemia, increased capillary permeability local tissue reaction and consequent changes occurring in inflammatory processes Nevertheless, there is ample proof that histamine either endogenous or exogenous, will provoke hyperemia and increased capillary permeability that these two phenomena play a significant part in inflammatory processes and that mast cells contain relatively large *quantities of releasable histamine and are almost* universally present in the connective tissues of normal structures [1, 108] and commonly occur at the site of local inflammation [633, 691] It has repeatedly been shown that the intradermal injection of a minute (1 to 1 or 2 million parts[88]) concentration of histamine speedily produces the triple response (1 5 to 3 minutes[88]) and the four cardinal signs of inflammation [390, 88] Nevertheless Ungar (1953) believes that this reaction does not prove that histamine is the only mediator of the inflammatory reaction,[484] but that it might be attributed to a more fundamental process' of which histamine release could be merely incidental [104]

A series of concepts relative to the relation of connective tissue including its active fibroblasts mast cell content and ground substance, to the onset and support of inflammatory processes lend significance to the importance of the part played by HA in particular and to the products of mucopolysaccharide (MPS) metabolism in general in these processes The opinion has been expressed that loose connective tissue is a primary site of inflammation[430] and the chief site of mast cell formation [693, 8]

Increasing interest is being shown in the part played by HA which is thought to be derived from mast cells [4] in maintaining normal capillary permeability by degradation of the MPS Recent work shows *that the* mode of action of cortisone and certain other antiphlogistic adrenocortical hormones is to produce increased capillary resistance[503] which is effected chiefly by suppressing the activity of hyaluronidase [3, 6] These and other observations on the vasomotor effects of histamine and serotonin and the significance of mast cells as the probable source of these substances certainly warrant further consideration

Capillary Dilation

Celsus (25 B C to 45 A D) clearly recognized the four cardinal signs of inflammation[6, 0] and in the absence of any knowledge of capillaries

of tissue damage that is caused chiefly by repeated episodes of necrosis or alternating degenerative and proliferative changes[661] especially in allergic or rheumatic reactions[1011] The initiating agent releases histamine and depletes the resident mast cells of the normal tissue The release of histamine is speedily followed by the sequence of hyperemia increased capillary permeability protein leakage marked edema and stasis These stages are generally recognized and have been confirmed by us as being conducive to mastocytogenesis as well as invasion by other exudate cells As chronicity develops vascularity and edema progressively decrease while mast cells increase in numbers[667] Apparently the condition of stasis and early necrosis stimulates activity of the progenitor cell to salvage certain components from the exudate and retain them as granules Thereby the stem cells differentiate into mast cells

HISTAMINE IN ALLERGY

Unquestionably histamine plays an important part in the initiation or as a concomitant of all allergic reactions Its mode of action as related to other factors is controversial The significance of the mast cell in allergenic reactions is chiefly that it is a rich and readily available source of histamine especially in the skin where these cells doubtless play a very significant role in dermographism and general urticarial demonstrations Several investigators have shown that mast cells are probably the chief source of the histamine which is generally conceded to be directly responsible for initiating local hyperemia that results in the formation of welts hives wheals and so on which may be diagnosed as urticaria in one or more of its many forms

Hypersensitivity

Failure of inactivation or sudden release of histamine constitutes a most important factor in allergic or hypersensitive reactions and anaphylaxis Dragstedt (1945) reviewed the literature on the evidence for and against histamine being related to anaphylaxis[1048] He concluded that histamine plays a significant part in anaphylactic reactions but that the mechanism of its release is not understood A number of writers subscribe to the idea that the cause of acute anaphylaxis is the sudden liberation of histamine or histamine like substances from the tissues as a result of the shocking dose of antigen[1301] Schachter (1953) showed conclusively that the release of histamine is a significant factor in the toxicology of acute anaphylactic reaction in the rabbit[841] The suggestion that histamine is involved in most if not all reactions of substances that provoke hyperergic inflammations is supported by Rocha e Silva s[867] statement that the contraction of guinea pig ileum produced by a prepartion of rat anaphylatoxin is also

the blood stream, and the blood stream receives heparin, histamine and/or other substances from the mast cells

It has been observed that *prolonged inflammatory conditions that are favorable* for increasing the numbers ("infiltration") of lymphocytes and plasmacytes are also favorable for increasing the number of mast cells. Further observations on exudates suggested that the protein content of edema fluid determines whether or not the edema fluid will be favorable for mastocytogenesis and the transformation of small lymphocytes into plasmacytes. The protein content of the extravascular fluid is most commonly related to conditions that favor the passage of the large protein molecules through capillary walls into a situation which detains the plasma protein commonly by the formation of a fibrin network. However, a part of the plasma passes on. These requirements are met in prolonged inflammation with the added advantage of the presence of abundant, similar materials released from cells as in delimited necrosis, under similar circumstances of stasis. Thus transudates *or edema fluid formed by stasis such as in cardiac or renal edema* in which the fluid has the lowest protein content of all edema fluids[100] and consequently low specific gravity[663] is not favorable for either mastocytogenesis or plasmacytogenesis.

The fixing and staining procedures that are used routinely by pathologists and many other students of inflammatory processes usually destroy the mast cell granules[594, 603, 88]. Thus the most reliable characteristic for identifying them is lost. Another point is that some investigators recognized an increase in the number of mast cells and tissue damage which was attributed to "repeated episodes of necrosis" in chronic inflammatory processes[663]. However, mast cells were considered to be merely "a type of infiltrating cell" and to have an unknown purpose[633, 663].

Stemmler (1921) observed that the normal population of mast cells is reduced in acute inflammation as well as in granulation tissue but that the number increases wherever mature tissue is being formed[894]. This observation supports the contention that the resident mast cell population is decimated early in acute inflammatory processes in response to the demand for *or otherwise provoked release of histamine and that no new* mast cells are formed until a sufficiently favorable exudate is provided. Halpern[390] states that all of the phenomena produced by injection of egg white "are borrowed by the typical inflammatory lesion." This statement is very significant in support of the idea that mast cells play an important part in inflammation, because injection of eggwhite or ovomucin especially into rats, provokes lysis of mast cells and liberates quantities of histamine[63, 391, 869, 896].

The mast cell population is reduced in acute inflammation[1, 633, 661] but increased in chronic inflammation in which there is an appreciable amount

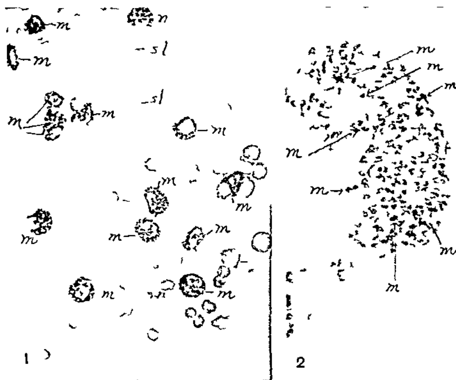


FIG 1 Mast cells (m) and small lymphocytes (sl) from peritoneal fluid of a normal hamster

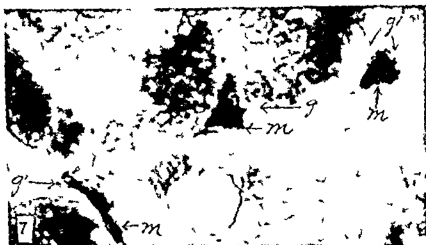
FIG 2 Mast cells (m) in an interrenal ganglion of hamster

due to release of histamine from the isolated organ? Schild[914] sets forth data to support his tenet that the release of histamine plays about the same role in allergic asthma of man as it does in anaphylaxis of guinea pig. Contrarily Code (1944) believed that histamine is not the fundamental factor in allergic reactions and anaphylaxis.[416] He states that the liberation of histamine is merely incidental to cell damage which is of primary importance in instigating these reactions. Eleven years later he[1?4] amplified this tenet somewhat by stating that the major portion of the histamine occurs in the serum which facilitates its activity, instead of being present in the white cell. Urbach and Gottlieb[1048] feel that the assumption that the substances which are usually accorded the role of instigators such as acetylcholine and histamine are not the cause but the result of antigen antibody reactions. Speirs[98] believes that the eosinophilia which follows antigen antibody reactions apparently is not caused by histamine heparin hyaluronidase or choline. Urbach and Gottlieb[1048] point out that neither blood nor tissue eosinophilia *per se* is conclusive evidence that an allergy exist. They add that allergies may be a result of increased stimulation of parasympathetic nerves and point to the fact that parasympathetic stimulating drugs favor allergization or tend to prolong an existing hypersensitiveness. Also a diet low in ascorbic acid has been considered to be conducive to allergies in both man and mammals.[1048] Histamine is probably not the substance responsible for shock even in animals injected with the substance[60] and probably is not the primary factor in traumatic shock.[416] Dragstedt (1945) expresses the concensus by stating that although histamine *is a definite factor in anaphylaxis no one appears to understand the* mechanism which releases it.[1048]

Allergic Response

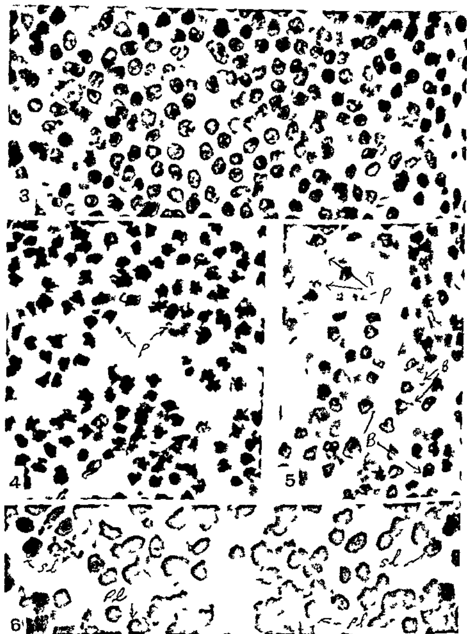
Perhaps the most plausible concept of anaphylaxis is that suggested by Urbach and Gottlieb.[1048] They citing the works of Code (1944), Wendt (1939) and others reject the explanation that anaphylaxis is the result of a simple histamine intoxication as being inadequate. They state that anaphylaxis is the result of a series of interactions of a number of biologically active substances of different types of tissue products such as choline epinephrine histamine and others which affect the chemical regulation of the autonomic effector organ and the autonomic nerve.[1048]

It is supposed chiefly upon indirect or circumstantial evidence that Lewis' triple response (characterized by a red line flare and wheal) insect bites irritants and various other forms of local damage to the skin liberate histamine.[61] Lewis (1927) thought that his triple response is identical with the reaction resulting from intradermal injection of histamine which causes the local dilation of capillaries and arterioles with





Figs. 7, 8 and 9 Mast cells (m) releasing granules (g) in the sublingual (mucus secreting) gland of the hamster (7), in submaxillary gland (8) and in the mesentery (9)

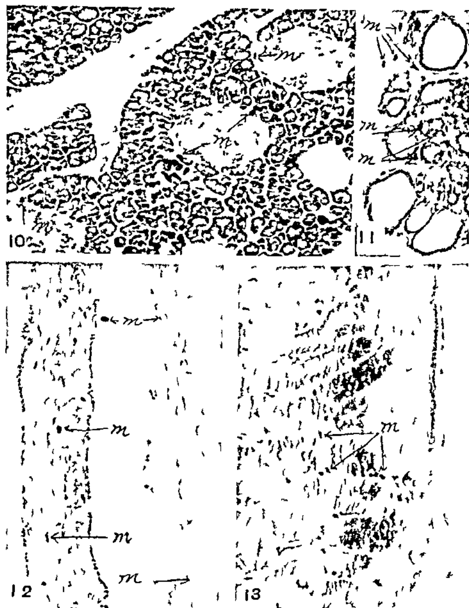


Fics 3 4 and 5 DNA (Feulgen method) in nuclei of small lymphocytes from a lymph node (3) spleen (4) and primary cells of a human tumor to low power (4) and nuclear budding (B) and partly depolymerized extranuclear DNA particles (P) (5)

Fig 6 Small lymphocytes (sl) and plasma cells (pl) showing cytoplasmic disintegration Toluidine blue stain



Figs. 14 and 15 Mast cells (m) in the mammary gland of a 66-day-old female hamster which received 60 000 IU of estrogen over 19 days (14) and in mammary gland of control female (15)



Figs. 10 and 11 Mast cells (m) in the islands of Langerhans of hamster (10) and in the thyroid gland (11)

Fig. 12 Mast cells (m) in a bovine synovial membrane

Fig. 13 Mast cells (m) in Brunner's glands in the duodenum of hamster

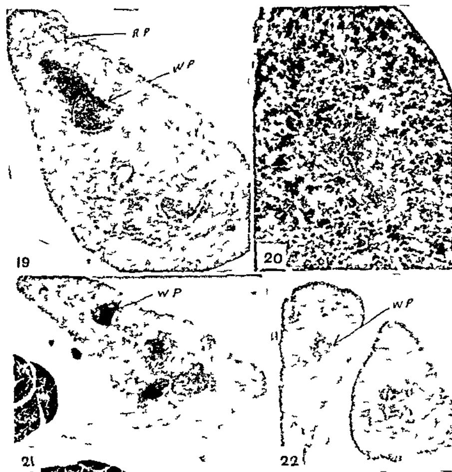
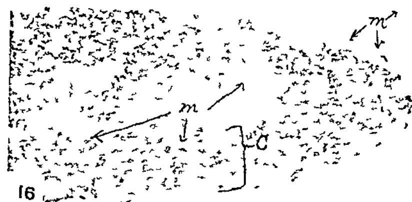
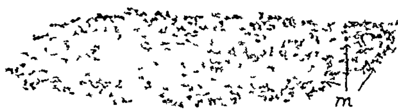


Fig 19 White (WP) and red pulp (RP) in the spleen of a normal adult female hamster (Plate #91 Ref 518)
Fig 20 Hematopoiesis in the red pulp of a hamster pregnant 12.5 days (Plate #91 Ref 518)
Fig 21 Spleen of a hamster that lost 33% of weight on a reduced diet (Plate #91 Ref 518)
Fig 22 Effect of cortisone on spleen of hamster All figures same magnification (Plate #4 Ref 206)





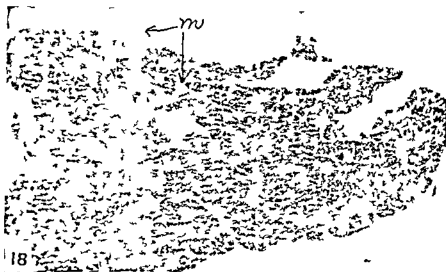


Fig 16 Mast cells (m) in thymus gland of normal 182-day-old male hamster

Fig 17 Thymus of a 100-day-old male hamster killed [illegible] days after total body x-irradiation of 995 r

Fig 18 Increase in lymphoid tissue in thymus in a 1[illegible]2-day-old male hamster killed 33 days after 995 r

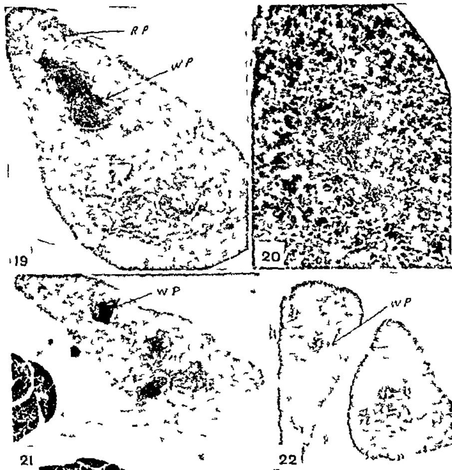


Fig 19 White (WP) and red pulp (RP) in the spleen of a normal adult female hamster (Plate #91 Ref 518)

Fig 20 Hematopoiesis in the red pulp of a hamster pregnant 12.5 days (Plate #91 Ref 518)

Fig 21 Spleen of a hamster that lost 33% of weight on a reduced diet (Plate #91 Ref 518)

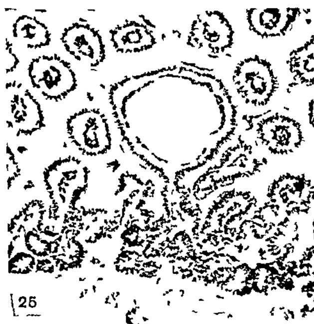
Fig 22 Effect of cortisone on spleen of hamster All figures same magnification (Plate #4 Ref 706)





Fig 23 Hematopoiesis (HP) in the red pulp of a hamster that had a sarcoma for 21 days

Fig 24 Spleen of a hamster that had implanted sarcoma for 23 days and was killed 142 days after complete extirpation of that sarcoma









Figs 25 to 28 Effect of excessive chronic administration of cortisone on the villi (25) the thymus (26) and lymph nodes (27 and 28) (Fig 26 and 27 Plate #1 Ref 206)

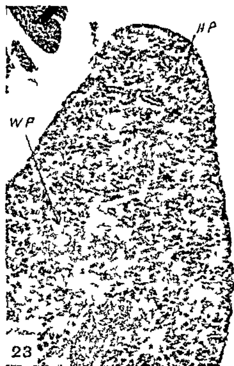




FIG 23 Hematopoiesis (HP) in the red pulp of a hamster that had a sarcoma for 21 days

FIG 24 Spleen of a hamster that had implanted sarcoma for 23 days and was killed 142 days after complete extirpation of that sarcoma

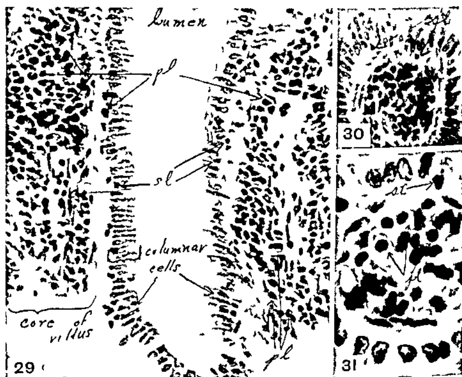
the escape of protein rich fluid.[1048] Rothman[83] points out that there is now available sufficient evidence to show that Lewis' "H substance is actually histamine." Riley[84] aptly attributes the ease with which this triple response may be elicited in urticaria pigmentosa skin lesions in man to the fact that lesions of this kind constitute a type of "shock organ" which is "rich in mast cells" and thus capable of releasing a quantity of histamine upon the least provocation. Contrarily, Perry[79] believes that the histamine responsible for the triple response does not come from mast cells. This statement would be rather difficult to prove or disprove experimentally, but in the light of the known mast cell content of histamine in human and other skin,[19, 301, 771] it is open to considerable skepticism.

There is probably a definite disturbance in the water balance and in certain enzymic cycles during the course of allergic diseases. There are inconsistent and non characteristic alterations in the chemical components of the blood, particularly with regard to amino acids, calcium, cholesterol, chlorides, magnesium, phosphorus and potassium content.[1048] Rusk and collaborators (1939) found that the serum potassium values were definitely higher in urticaria (23.4 mg/100 ml), in acute asthmatic attacks (24.4 mg/100 ml) and in the asymptomatic period in bronchial asthma (23.6 mg/100 ml) than in healthy human subjects (19.5 mg/100 ml).[1048] Epinephrine produced relief in these patients, but it did not significantly alter the serum potassium level. Various investigators have found that the blood histamine level is higher in asthmatics than in normal individuals.[1083] Nevertheless, Rose (1954) points out that the release of histamine is not the sole cause of asthmatic symptoms.[1053]

Histamine in Shock

The part played by histamine in several types of shock is not clearly demonstrated. Especially in man, the course of shock is not constant but may be strikingly diversified.[869] Lung emphysema in certain human cases and in guinea pigs, liver congestion which is common in dogs, or venous congestion caused by the dilation of the right heart, as in the rabbit, are effects of anaphylaxis.[889] Since shock is a condition of acute peripheral circulatory failure,[239] there are grounds for suspecting that histamine is either associated with the cause of shock in certain types or with the effects of shock in those types not otherwise explained. Thus histamine may be associated with, but possibly not the cause of, primary shock. Also it is conceivable that factors other than neural or histaminic may be responsible in certain types of shock. Briefly, shock supervenes whenever a sufficient amount of blood plasma is lost from the circulation, usually by pooling, hemorrhage or transudation.[39]

Halpern[331] states that the intraperitoneal administration of a histamine



Figs 29 to 31 Plasmacytes (pl) and lymphocytes (sl) in core of villi and in columnar epithelial cells

tions, which is accompanied by potassium and pH changes Acidosis of a transitory nature has been observed in both animals and men following a variety of systemic stressors including anoxia burns electro shock, 'gravity shock' and medical shock'[9] However, nothing is said about mast cells as a source of the histamine that is known to be released in relation to some of these conditions especially burns[653] and the resultant increased capillary permeability Menkin[65] shows that, with few experimentally produced exceptions in dogs similar changes take place in the inflammatory process Other investigators[599, 631, 663] have recorded concomitant changes in local mast cell populations but neither mast cells nor metachromasia is listed in the index of either Selye s[9] or Menkin's[65] monograph

Mongar and Schild (1952) point out several ways in which the effects of anaphylaxis and certain simple chemical bases are similar in releasing histamine but that their mode of effecting the release of histamine differs[904] Reuse[341] maintains that anaphylaxis is due to the release of histamine Parrot (1942) and Mongar and Schild (1955) believe that in anaphylaxis the release of histamine is a process that requires energy and that it may be blocked by various metabolic inhibitors including iodoacetate or lack of oxygen[904] However chemical releasers such as acetylamine or compound 48/80 do not require the provision of energy but are activated by iodoacetate or the lack of oxygen as shown by minced lung of guinea pigs *in vitro*[904]

Wilander[1094] isolated sufficient amounts of heparin from the plasma of dogs in peptone shock to account for the incoagulability of the blood in anaphylactic or peptone shock He reported that protamine completely allayed the anticoagulant effect of the heparin in the dogs He believes that the mast cells during shock empty their granular contents into the blood and that blood clotting was not inhibited in hepatectomized dogs but that shock plasma recovered after being passed through the isolated liver inhibited coagulation

Antihistimine drugs operate by competing with histamine for the normal histamine receptive sites By this means antihistamines prevent the appearance of histaminic effects[407] Such a simple explanation of histamine antagonism may be valid for some but not all histamine antagonists because these substances are also histamine releasers and many of them when used in concentrations sufficient to antagonize anaphylaxis of smooth muscle independently produce a bronchoconstrictor action which reduces their effectiveness in treating bronchial asthma[904] Similar effects of antihistamine drugs on histamine which produced anaphylactic contraction of smooth muscle have been explained as a phase of the contraction which is not antagonized by the antihistamine[413]

liberator (dextran, 30 mg/100 g) to adrenalectomized rats, provoked severe dyspnea, prostration with acute vascular collapse and death of all the animals within 20 minutes Ovomucoid (1 mg N/100 g) produced similar symptoms, but the rats lived a maximum of 2 hours Intravenous injection of these histamine liberators greatly increased their toxicity Helpern concluded that the basic and major disturbance produced by the administration of dextran or ovomucoid is "damage of the small blood vessels, which is reflected by an increase in capillary permeability" that results in the formation of a *characteristic edema* which has a high protein content and a severe hemoconcentration He further indicated that histaminic action was involved by finding that edema and almost all the symptoms caused by dextran can be prevented in intact rats by injection of the synthetic antihistamine promethazine or Phenergan Bennati and Patetta[67] found that Benadryl protected animals against lethal histamine shock but not against peptone shock

Glenn Gilbert and Drinker (1943) found that experimental burns of the foot provoked the escape into the tissues of quantities of plasma which approximated a third of the total volume of blood [663] Such conditions of shock certainly indicate the current concept of histaminic activity and probably involve heparin as well However the work of Rowley and Benditt[884] indicates that serotonin plays an important part as a causative agent in cases of profound edema especially if mast cell damage is involved as would be expected in extensive skin burns In the case of the experimental burns of the foot cutaneous and subcutaneous mast cells would be expected to release relatively large quantities of histamine and heparin Incidentally the fact that decreased coagulability of the blood is one of the functional effects of shock,[663] suggests increased release of heparin, a fact which has been verified in dogs anesthetized with Dial vagotomized and injected intravenously with peptone [34]

Histamine shock is thought to differ from anaphylactic shock in the absence of the reduced body temperature and in increased blood coagulation time, both of which are characteristic symptoms of anaphylactic shock [1048] A marked increase in coagulation time was observed in 4 guinea pigs killed while in profound anaphylactic shock produced by horse serum [535] Also it is stated that arginine prevents the lethal effects of administered histamine but not of anaphylactic shock whereas heparin inhibits anaphylaxis but is incapable of inhibiting histamine shock [1048]

Rocha e Silva (1947) presents another complication by pointing out that in anaphylactoid conditions fibrinolysin releases histamine and heparin from cell [9] but it is not clear whether or not mast cells are included Selye[8] notes that various stresses alter the water balance chiefly by disturbing the intracellular protein intercellular sodium rela

tions, which is accompanied by potassium and pH changes Acidosis of a transitory nature has been observed in both animals and men following a variety of systemic stressors, including anoxia burns electro shock, 'gravity shock' and "medical shock'[972] However, nothing is said about mast cells as a source of the histamine that is known to be relea ed in re lation to some of these conditions e pecially burns[663] and the resultant increased capillary permeability Menkin[68] shows that, with few experi mentally produced exceptions in dogs, similar changes take place in the inflammatory process Other investigators[99 834 663] have recorded con comitant changes in local mast cell populations, but neither mast cells nor metachromasia is listed in the index of either Selye's[972] or Menkin s[68] monograph

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Martin and colleagues (1949) reported that flavonoid compounds effectively inhibited histidine decarboxylase in tissue cultures.[647] This result probably led them to suggest that the anti anaphylactic effect of the flavonoid compounds used is the result of the ability of these compounds to prevent the formation of histamine from histidine by inhibiting the enzyme decarboxylase.

Histaminopexy

Histamine fixation by experimental methods is a comparatively undeveloped field. Theoretically at least it should be possible to inject locally, intravenously or intraperitoneally some substance which would prevent the release of, or inactivate, liberated histamine by binding it in some way to molecules in the plasma, tissues and/or to a substance included in the injection.

The idea of histaminopexy loses some of its aspects of impossibility when viewed as a reversal of histamine release processes in which loosely bound inactive histamine[669, 670, 1045] is activated. It is not known whether this principle would be applicable to endogenous histamine, because exogenous histamine is not incorporated by cells. Nevertheless, Laborde, Parrott and Urquia (1953) showed that normal human serum fixed about one third of the exogenous histamine (histamine dihydrochloride 10^{-6} and 5.0 per cent dialyzed human serum) in a solution, but that sera from most allergic patients showed no reduction in activity of the histamine by the sera as indicated by the guinea pig ileum test.[784] Parrot and Laborde[783] found that the histaminopexic activity of sera was controlled to a significant extent by adrenocortical and adenohypophysial hormones, and was reactivated by cortisone.

The statement that anti histaminic drugs operate by blocking the response of receptors to the stimulating effect of free histamine[841] suggests that the beneficial results of these drugs is not due to their histaminopexic activity.

Bloschko[86] believes that liberated histamine exerts its biological action after passing from the circulating and tissue fluids by again being bound to specifically histamine sensitive patches on the effector cells' surfaces. If this is proved to be the mode by which endogenous histamine operates in allergic reactions, then any practical means of desensitizing these histamine sensitive cell surface patches or to cause them to repel the invading histamine would certainly be well worth considering for use in prophylactic or therapeutic treatment.

A rather novel idea of a contributing factor in histamine control is set forth by Parrot and Laborde.[783] These authors describe a histaminopexic gamma globulin like substance which, subsequent to removal by dialysis of an anti histaminopexic albumin linked serum fraction (Fraction V

of E J Cohn), reduced the histamine activity of normal human serum about 33 per cent and was at least equally effective on normal sera of the cat, dog guinea pig (average of 10, 39.2 per cent) mouse and rat (average of 8 38 7 per cent) However, this histamine fixing substance failed to bind any of the histamine in the sera from most of 51 allergic patients who were suffering from asthma angioneurotic edema chronic arthritis eczema, duodenal or exudative and gastric ulcers hay fever, migraine, rheumatic fever or tuberculosis exudative [783] Parrot (1955) showed that adrenalectomy deprived rats of this histaminopexic substance, but that it was restored by the administration of cortisone [900] and that deficiency of ascorbic acid also deprived serum of its histaminopexic action [78]

Reuse[841] believes that the beneficial effects of anti histamine in ana phylaxis are the result of these drugs inhibiting the effects of liberated histamine on effectors ' as is indicated by muscle tissue *in vitro* Ungar[1045] cautions that there is a possibility that proteose activation and histamine release are parallel phenomena that are due to a common cause, even though it is known that inhibition of proteolysis suppresses the release of histamine [101]

CORTICOSTEROIDS

At least 29 corticosteroids have been identified in extracts of the adrenal cortex, but only about six of these have been shown to be active [10 6] Elkinton and Danowski [79] show that adrenocortical steroids in excess generally disturb the body fluid balance by causing sodium retention and potassium deficits accompanied by other significant changes in the salt water balance sugar ionic and other body fluid disturbances However very little or no consideration is given to histamine

Adrenocorticotropic hormone (ACTH) cortisone and epinephrine (all anti phlogistic hormones of Selye[9] are antagonistic to histamine These substances decrease capillary permeability and the formation of inflam matory exudate However DOC (compound S desoxycorticosterone Desoxycortone) and DOCA (DCA desoxycorticosterone acetate or pro phlogistic hormones of Selye) like histamine cause capillary dilation increased permeability and increased exudate and thus either have no effect on or actually favor histaminic action Cortisone either adminis tered to or produced within the animal following the administration of ACTH is thought to be converted into hydrocortisone within the body be fore it becomes physiologically active [8]

ACTH (Adrenocorticotropic Hormone)

It is generally conceded that ACTH which is an anterior hypophyseal hormone and cortisone have nearly identical effects because ACTH acts by stimulating the adrenal cortex to produce corticosteroids, especially

cortisone[358] Conn and co workers (1931) have indicated indirectly that ACTH administration produces 17 hydroxycorticosterone (compound F) as the mediator of its action[892] Menkin[683] shows that numerous investigators have found that ACTH is as effective in suppressing increased capillary permeability in inflammation in adrenalectomized rats as it is in normal rats He is convinced that ACTH acts directly in decreasing permeability of the capillaries by suppressing the action of exudin Menkin[683] further supports the idea that these two hormones have different effects by stating that ACTH has no effect on leukotaxin induced diapedesis, but represses the effects of exudin in producing increased capillary permeability, cortisone has no effect on exudin, but inhibits the diapedesis ACTH in excess has been found to induce freckling and pigmentation similar to that occurring in Addison's disease, but excessive dosage of cortisone does not produce this effect[79] Other less clear cut differences in the effects of ACTH and cortisone have been reported

Cortisone

The chief basis for the early recognition of cortisone as a 'wonder drug for the treatment of various conditions that involve capillary hyperenergy may be attributed to its ability to decrease capillary permeability There are several possible means by which cortisone may decrease capillary permeability in normal as well as in histamine induced and other edematous conditions Romani[874] calls attention to the fact that there are numerous contradictions in the literature on the effects of cortisone on mast cells Actually the recorded effects range all the way from producing a marked increase in number[874] to the destruction of mast cells[0, 150]

EPINEPHRINE AS INITIATOR

There is the possibility that immediately beneficial effects of cortisone in reducing conditions which result from excessive amounts of histamine "H substance" leukotaxin and/or similar agents which increase capillary permeability may be due to cortisone which increases the release of epinephrine which in turn constricts arterioles[964] and thus decreases capillary dilation and permeability by reducing the volume of blood passing through the arterioles and precapillaries Selye[9] suggests that competition for the same amino oxidase system may account for the potentiated pressor action of epinephrine derivatives in controlling hyperemia and permeability However, multiple factors in unknown sequence are probably involved One of these little appreciated factors may be 5 hydroxytryptamine serotonin Spategift of Freund)[9] which is stored in mast cells[6, 576, 884] in blood platelets[978] and probably in other cells It is claimed that human

blood contains about 0 1 γ/ml of serotonin[684, 978] However, it appears at present that very little is known about the specific relations of serotonin to either inflammatory hyperemia or to the effects of either epinephrine or cortisone on capillary permeability However, evidence obtained from more than one source "makes it very likely that 5 hydroxytryptamine is the edema producing agent released by substances which damage mast cells"[884]

EFFECTS OF CORTISONE

Several investigators have reported that cortisone inhibits leukotaxin[68] and hyaluronidases by forming a complex heparin lipoprotein which acts as a non specific anti hyaluronidase With this exception, the mechanism of the anti phlogistic action of cortisone appears to involve the sulfhydryl groups[874] Moderately prolonged treatment with cortisone causes various metabolic changes, but it is debatable whether polymerization of HA is altered by cortisone HA increases the resistance of ground substance to the spread of inflammation and thereby plays a very important part in controlling the edematous effects of increased capillary permeability -[1] Thus, it appears that enzymic systems may play important roles in histamine epinephrine control of the motivation of arterioles and their capillaries

Cortisone also suppresses the formation of new capillaries as in wound healing,[1, 464, 66] and in the vascularization of tumor skin and other grafts[15, 566] Cortisone may have a direct anti mitotic effect in skin grafting as well as the commonly recognized indirect effect by inhibiting the process of vascularization of the graft[66] Apparently the mode of action of cortisone in suppressing capillary growth and permeability is through its antagonism of histamine This process may be abetted by other substances reported to be active in increasing capillary permeability in normal and inflammatory conditions

Cortisone probably as the hydrochloride[8] is a potent anti inflammatory agent which is supposed to have multiple means of producing this effect In fact there are three means by which cortisone suppresses histaminic activity One method is by blocking one or more enzyme systems but the particular enzyme system blocked is unknown[8] Most investigators believe that histamine is produced by decarboxylation of histidine by histidine decarboxylase[69, 900, 1084] Helpern[391] states that cortisone inhibits the biogenesis of histamine the vulnerable link for which would be histidine decarboxylase The enzyme histaminase destroys histamine by catalyzing its oxidation[577] Cortisone is believed to suppress histamine by interfering with its production and by aiding in its destruction Halpern[390]

adds another perspective by suggesting that cortisone acts by interfering with the metabolism of tissue histamine, more specifically by preventing the degradation of the "combined" form to the "free" form of histamine

Ungar[104] believes that a number of anti inflammatory drugs, including cortisone act by inhibiting the fibrinolytic system *in vivo* as well as *in vitro* Thus, he concludes that all anti phlogistic hormones accelerate the fibrinolysin anti fibrinolysin reaction Zweifach (1953) states that va o dilation with its attendant phenomena can be produced by numerous bio logical substances many of which are formed by cells He sees no need for any proteolytic type of enzymic reaction [104] Thus, it may be inferred that there are multiple points other than the e in the fibrinolytic system which can be blocked by cortisone or other anti inflammatory substances to prevent the release of histamine and/or increased permeability of the terminal vascular bed

Desoxycorticosterones

It is often difficult to determine just which preparation of DOC was used, because variable terms and abbreviations are used by different writers It appears that under comparable conditions the various preparations of the DOC all properly belong in Selye's[9, 1] (1949) group of prophlogistic hormones, that is they aid or stimulate histaminic vasodilation and in creased capillary permeability The names of some of these preparations with their synonyms in parentheses as nearly as can be determined, follow desoxycorticosterone (compound S (?) Desoxycortone DO DOC) and desoxycorticosterone acetate (DCA and DOCA Acetate Deoxycortone acetate and Percortin—proprietary brands)

DCA is the form of DOC most often employed in experimental and clinical work In general DCA exerts a more pronounced effect on certain species than on others Although all rats are susceptible to the hyalinizing effects of DCA some strains are more susceptible than others, the fowl and rat apparently represent the species most ensitive to its hyalinosis pro ducing effects [9] This species su ceptibility may be largely responsible for the seemingly contradictory results reported by certain investigators

DCA promotes inflammation and wound healing apparently as a result of its phlogistic reaction and thus it stimulates the proliferation of fibro blasts and encourages the deposition of collagen [9] Apparently the bene ficial effects of DCA on wound healing and its aggravating effects on in flammatory processe are es entially similar to the effects of mast cell products That is both increase capillary permeability and afford an ade quate supply of blood borne ub tances that are necessary for the forma tion of fibroblasts collagen cicatricial ti sue and other tissues such as connective tissue adnexa, neural and vascular structures This point be

comes more obvious when it is recalled that cortisone and ACTH inhibit inflammation and wound healing by suppressing the processes which DCA and mast cell products promote

Desoxycorticosterone diethylacetate appears to be an exception to the general rule because this substance is slow acting Unlike DOCA Acetate it decreases the amount of inflammatory exudate in croton oil granuloma pouches in rats However, it was found to be only about 20 to 30 per cent as potent as hydrocortisone [8 3]

Percortin a proprietary preparation of DCA which was available in the early 1940's has been reported to be anti inflammatory in action but the anti phlogistic effects of batches of this substance may not have been due to pure material [683] This conclusion was further supported by the fact that the substance in crystalline form obtained from another source has 'no anti inflammatory effects [683] However conditions of the experiment may have been such that the expected phlogistic results were not obtained The work of Skelton [947] in which he used antihistamines in a futile effort to antagonize the effects of DOCA Acetate on castrated unilaterally nephrectomized salt treated rats supports the results obtained by the use of Percortin Also he reports that under the conditions of this experiment it was found that the antihistamines Phenergan Benzodionole and Trimeton afforded no significant protection to those rats against either the angiotoxic or nephrotrophic effects of DOCA Acetate as seen in the heart kidney and arterioles in the pancreas

7

Heparin and Hyaluronic Acid

Evidence that mast cells play an important part in supplying hyaluronic acid (HA) as well as heparin, is increasing Mast cells appear to be firmly established as a very important source of heparin,[366, 00, 6, 990, 1085, 1094] but the relations of mast cells to HA production are not so well established, possibly, because work in this field is very recent The relations of mast cells to the production of HA, present so many possibilities that it is very difficult to determine just how and to what extent the mast cell is involved Histochemical methods are inadequate in determining the origin of sulfate free HA because AMPs that contain the sulfate group are usually present also Nevertheless, it appears that mast cells influence the production, and perhaps the activity, of HA It is possible that mast cells influence HA production indirectly by altering capillary permeability, for certain investigators suggest that connective tissue ground substance and/or synovial fluid both of which contain HA [1, 24, 60, 686] is formed by fibroblasts [10, 7, 1089] Others believe that it is transferred directly from blood plasma [548] Burkel (1952) suspected that HA was a prestage form of heparin [995] Asboe Hansen [1] however suggests that the mast cell secreted precursor of HA may resemble heparin

Since heparin is an AMP[500, 6, 1, 990, 108, 1094] and since HA is usually classed as a mucopolysaccharide (MPS), [1, 4, 60, 10, 7] the origin of these two substances may be more closely related than is commonly supposed The above information indicates that heparin is an anticoagulant which does not alter the consistency of the vascular endothelial surface cement film[667] and that HA in solution acts as an important lubricant [60] The possible combinations of these two factors may be essential for the optimal performance of a number of processes

HEPARIN

Mast cells are considered a source of heparin by many investigators who believe that in most cases there is a correlation between the mast cell population and the heparin content of the tissues [500, 6, 7, 7, 0, 8, 5, 990, 108, 1094] However other investigators failed to find a correlation between heparin content and mast cell population in certain organs of rats and rabbits [318] Suffice it to say at this time that mast cells are not the only source of heparin For instance, Behrens and Taubert (1952) extracted heparin or a

biologically and physicochemically heparin like substance, from isolated mast leukocytes of the horse[649] Charles and Scott (1933) demonstrated the presence of heparin in all tissues which included the blood heart liver, lung muscle spleen and thymus but it was most abundant in liver, lung and muscle which contained about equal amounts[181] The amount present in the blood serum was almost negligible[151]

It is assumed that heparin occurs in the blood in combined form and that 'the existence of free heparin in normal blood appears to be unlikely'[706] Mota[7 0] concurs in this statement Monkhouse and Jaques[06] recovered 40 per cent of the heparin added to blood (even when the concentration was as low as 0 007 mg/100 ml) but were unable to obtain enough heparin from the blood of normal cattle dogs and people (to which no heparin had been added) for an estimation From these coagulation tests they concluded that free heparin was either absent from the normal blood of cattle dogs and men or was in a concentration less than 0 1 μg/ml

Heparin is a naturally occurring rather strongly acid anticoagulant[303, 416, 1084] It is considered the most highly ionized polymeric substance synthesized by the animal body[37] Heparin which is similar to mucoitin sulfuric acid[1084] is a mucopolysaccharide (MPS) that contains sulfuric acid[37] The exact chemical nature of heparin is not known but more than one ester sulfate group are present and the molecular weight is of the order of 17 000 which would make it unlikely that the degree of polymerization exceeds 200 monosaccharide units[37] In general the activity of preparations of heparin is related to its ester sulfate content[537] However commercial preparations of heparin may contain 10 per cent inactive polysaccharide which is chiefly if not exclusively residue acetylhexosamine After removing this 10 per cent of inactive amine groups by electrophoresis the remaining 90 per cent which contains no acid group other than sulfuric and uronic may be substituted with sulfuric acid forming special groups of $NH—SO_3H$[659] The suggestion has been made that beta heparin bears the N acetyl groups and that alpha heparin does not contain any of these acetyls[537] In general the heparins are considered AMP constituents of mast cell granules[790] Jorpes and Bergstrom (1937) point out that since the amino sugar found in purified heparin instead of being galactosamine which is characteristic for chondroitin actually is glucosamine which is a component of the closely related mucoitin heparin is to be considered a mucoitinpolysulfuric ester[6 1] Sylven[1001] and MacIntosh[6] also state that tissue mast cells or the granules of mast cells are a source of native heparin However Ham[393] suggests that mast cells are associated with connective tissue and that it would appear that there is some relationship between heparin mast cell granules and sulfated amorphous intercellular substance which is not yet clear since both the mast granules and the sur

rounding intercellular substances have similar, amorphous sulfated components Sylven (1951) and his co workers (1950) showed, by means of differential extraction, that heparin is not within the mast granule, but merely forms an amorphous, probably protein bound, coating on the surface of the granule[6 7 89]

The confirmation of Noback and Montagna's[745] finding alkaline glycerophosphatase in mast cell granules and Wislocki and co workers[1107 1108] finding acid glycerophosphatase also present in them broadens the possible field of interrelations and functions of these granules Ham[393] doubts if mast cells are capable of producing enough heparin to maintain the fluidity of the blood under normal conditions and suggests that the normal function of mast granules might be to maintain the fluidity of the fibrinogen which has escaped from blood capillaries into the tissues where otherwise it would likely undergo gelation

Heparin is a powerful anticoagulant It also protects thrombin from oxidation by oxygen, but loses this function in the presence of toluidine blue[77] Its mode of action in this capacity apparently is in conjunction with a plasma protein, probably an albumin, to form an antiprothrombin which inhibits the conversion of prothrombin to thrombin[1084] When heparin is combined with some other plasma albumin fraction, it may form an antithrombin instead of an antiprothrombin, which inhibits or prevents the action of thrombin upon fibrinogen[1084] The question of heparin antagonizing the release of histamine arises repeatedly, but its mode of action apparently is not well understood Dragstedt and co workers (1942), by the use of antigen proteose and trypsin demonstrated that heparin prevents the release of histamine[970]

There is strong evidence that heparin as well as histamine is released from mast cells by histamine liberators[7 0 8 1 853 8 7 869 990 1095] In anaphylaxis and peptone shock the blood is incoagulable due supposedly to large quantities of the anticoagulant heparin which is poured into the blood under these conditions[416 1048 1084] The conclusion that mast cells are a source of heparin should be correlated with the more recent literature which shows that a number of forms of AMP other than heparin inhibit coagulation and decrease viscosity For example AMPs from the umbilical cord have been described by Sundberg and co workers[996] as containing a factor that inhibits coagulation before and after treatment with hyaluronidase

Heparin was discovered in 1916 by McLean, a student of H W Howell[303 6 1] and was named by Howell and Holt in 1918[964 1084] Heparin appears to be the first and most widely accepted physiologically active substance attributed to mast cell granules[303 00 1094] Mast cells (presumably because of the granules) were shown to be chemically related to mucin as

mucoid degenerating cells' by Raunitz as early as 1883 and were considered by Harris in 1910' to elaborate the mucin of the connective tissues[693]

Three or possibly more kinds of heparin are recognized, and various writers suggest that different species of mammals also have different heparins[40, 416, 651] and that the same structure in different species has different amounts or strengths of heparin[416, 500, 651, 1094] These differences may be related to the fact that, in general, the activity of a heparin preparation is related to its ester sulfate content as was shown by the fact that a sodium salt preparation which was highly active contained 13 6 per cent of sulfur[537]

Another source of confusion was reported by Adams who found that some preparations of heparin contain sufficient amounts of conjugated histamine to mask the results of the experiment He found that 1 part of acetylhistamine to 7000 parts of heparin by weight was ample to confuse the results of his experiments[379]

Heparin is released from the tissues and blood cells by or during, anaphylactic shock[416, 1048] Compound 48/80 and probably most of the other histamine liberators liberate heparin as well as histamine from mast cells[39, 717] Heparin may be inactivated by proteins especially the basic ones such as protamines[33, 416, 1084] which may readily combine with the heparin[416, 1084]

Occurrence of Heparin

The presence of heparin rather than other forms of AMP in the granules of mast cells has not been unequivocally established Nevertheless there is usually a surprising correlation between the heparin content and the mast cell population of structures[60, 85, 501, 108, 1094] In beef lung which is an important commercial source of heparin mast cells are numerous in the parenchyma but are usually large and very numerous just under visceral pleura[1095]

Many investigators believe that mast cell granules are coated with or contain and/or secrete heparin[1, 60, 88, 9, 1079, 1095] but others qualify this tenet in many ways Jorpes Werner and Aberg (1918) consider mast granules to be heparin precursors because the granules stain metachromatically and usually are Periodic Acid Schiff (PAS) positive in human tissues[6] Despite the mass of evidence indicating that mast granules are covered with contain or actually are heparin Compton[181] cautions that proof of this tenet is lacking Iaff and Bloom[787] state that the metachromatic substance liberated by the granules is presumed to be heparin or some form of it Weiss[1074] states that mast cells probably produce heparin and he attributes the basophilia of their granules to the sulfate groups present in heparin Holmgren and Wilander have been credited with having advanced

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duct forms "almost a drainpipe leading from liver to blood and thus affords "the most likely route by which heparin gets round into the blood lends support to the idea that much of this thoracic heparin may have had its origin in mesenteric mast cells [53]

It has also been suggested that mast cells in the sinuses of lymph nodes may prevent the formation of fibrin and clotting by "discharging their heparin containing granules' into the sluggishly flowing sinus lymph especially in conditions such as lymphadenitis [9 4]

Paff Bloom and Reily [788] employing tissue culture methods, were unable to confirm Downey s finding that metachromatic granules or other metachromatic substance occur in the nucleus of cancer cells Thus they do not support his conclusion that metachromatic granules of tumor cells are derived *from the nucleus However in the cytoplasm of two well differentiated* mast cell tumors of dogs they found fine granules that failed to stain metachromatically, which they considered to be immature mast granules and large, mature granules which were strongly metachromatic Thus they confirmed results reported by Oliver Bloom and Mangieri[754] that there is a correlation between the depth of metachromasia and the size of the granules of the mast cells and the amount of heparin contained in the tumor tissue These investigators are reasonably certain that the massive heparin content' of the well differentiated mast cell tumors that they used proved Wilander s[1094] and Jorpes [500] contention that heparin is derived from mast cells and that the quantity of heparin is correlated with the number of mast cells present Other investigators have verified these findings[486 1085] so that the idea is generally accepted that there is a correlation between *the ripeness of mast cell granules (state II of Riley, 1953)* depth of metachromasia and amount of heparin in mast cell tumor tissue This correlation appears to apply to non neoplastic tissues also

Several investigators presume that mast granules produce both heparin and HA [3] Others believe that there is a definite correlation between the number of mast cells present in a tissue and the heparin content of the tissue[404 500 679 7 4 8 1 8 7 108] or of a mast cell tumor [7 4 8 108] This idea is furthered by several writers in the fields of biochemistry pathology pharmacology and physiology A different view is taken by Hedbom and Snellman [414] who believe that the heparin bearing native complex which is probably a heparin lipoprotein, is very likely located not in the large granules but within the intergranular cytoplasm They suggest that the heparin observed by investigators working with isolated large granules had been precipitated *on the surface of the granules* probably as a result of these workers failing to suspend the homogenate in a sugar or salt solution, and neglecting to control the pH of the final mixture sufficiently accurately Also a number of investigators believe that heparin as such

the idea that mast cells elaborate and secrete the specific MPS, heparin[990, 1108] Jorpes (1946) thought that mast cells produce the heparin for the body and that the heparin is discharged directly into the blood[99] Likewise, nearly all of the earlier as well as some later investigators believe that heparin, or a heparin like substance, is synthesized by and stored within (or attached to) the cytoplasmic granules of tissue mast cells[303, 468, 00, 5, 4, 754, 767, 8, 3, 8, 5, 954, 990, 1085, 1094]

Studer[990] believes that the heparin content of organs of the various species of animals parallels the mast cell content Thus, his statement supports and extends Holmgren and Wilander's (1937) finding that the heparin content of Glisson's capsule of the liver is related to the number of mast cells within it[99] That mast cells store heparin or its components may be deduced from the work of Asplund and associates (1939) and Frick (1950)[99] Schurer (1946) observed "filling of the mast cells and an increase in size and number of granules' (which stained normally) following the parenteral administration of heparin[995]

Heparin inhibits mitosis,[416] but very little has been known about the method by which it operates until recently Roth (1952) stated that heparin is an inhibitor of both ribonuclease (RNAase) and desoxyribonuclease (DNAase),[769] and Paff and co workers[769] have shown that inhibition of these enzymes is the means by which heparin inhibits mitosis of fibroblasts in cultures of embryonic chick heart

A new function of heparin has recently been postulated Mast cells especially those skirting' small blood vessels (state I of Riley, 1953) are reported to supply a metachromatic substance which is considered heparin, that combines with the cement granules in endothelial cells following vascular injury to form a gelatinous, heparin containing film The presence of this film protects the vessel from the formation of thrombi by virtue of its strong negative charge[66, 666] This condition prevents leukocytes and platelets from being attracted with the consequent organization of a blood clot at the site of injury[663, 666]

Numerous functions in addition to its anticoagulant properties are now attributed to heparin and to mast cells Some significant clinical and experimental investigations are being conducted to determine the significance of the relations of naturally occurring heparin to growth processes, particularly as an inhibitor of neoplastic growth in the formation of connective tissue ground substance as an anti shock agent and in arteriosclerotic vascular changes[990]

An abundance of mast cells is usually reported in the mesentery[693] However, no one has seriously considered the possibility that mast cells in the mesentery could be a source of AMPs for the production of heparin in the liver and blood stream Riley's[855] insistence that in the dog the thoracic

cerning the cause of the wide variations in number of mast cells in the wall of blood vessels in man Sundberg[995] gives various other reasons, including fairly great variations which occurred among persons dying "so called violent deaths" indicating that he does not attach particular significance to infectious conditions and diseases as the cause of the variations in average mast cell counts in the wall of large veins Similarly the probable increase in atheromatous deposits and/or the appearance of arteriosclerosis with advanced age makes it difficult to quantify the effect of advanced age on the mast cell population of vascular walls especially of the aortic wall

Hjelmann and Weghus (1950) observed that mast cells increased around experimentally produced thromboses and hemorrhoids[995] Hellstrom and Holmgren (1947) believe that a decrease in the number of mast cells in the wall of veins in elderly people may be related to the increased incidence of thrombosis in these individuals[97] Further intimation that mast cells appear to be related to human hemorrhoids is supplied by Hjelmann (1954) who reports that the number of mast cells in the wall of dilated hemorrhoidal veins was increased over that of controls[1091] However it is not clear whether the mast cells functioned in the formation of hemorrhoids by liberating histamine or in the resorption by liberating heparin

Clearing Hyperlipemia

Heparin plays important roles in the storage and general metabolism of fat and in clearing hyperlipemic and hypercholsteremic blood and lymph The daily intraperitoneal injection of 2 0 mg of heparin reduced the amount of fatty degeneration in the liver by 60 per cent and the formation of aortic atherosclerosis by about 40 per cent in cholesterol fed rabbits[690] However, several salts were found to inhibit *in vitro* clearing by post heparin plasma up to 80 per cent *in vitro*[44] Heparin has also been shown to inhibit the action of hyaluronidase *in vitro*[47]

Hyperlipemia or hypercholesteremia lipemia which is more properly designated hyperlipemia is a condition in which the total blood lipids exceed 1 0 per cent in human blood[444] Hypercholesteremia in its various groups usually shows atheromatous changes[444] whereas hyperlipemia and hypercholesteremia apparently are concomitant in subchronic conditions and both have multiple diagnostic and clinical significance[444] Administration of heparin results in the reduction of excessive amounts of lipid and cholesterol in the blood and lymph but its mode of action is not well understood However it has been shown that the size of the molecule of lipoproteins is reduced by heparin[99] It seems probable that insufficient plasma heparin may be a significant etiological factor in atherogenesis and sclerosis[98]

is not stored in the mast cell granules, but that these granules contain a precursor of heparin, or synthesize and store fundamental AMPs which are essential in the formation of heparin and other substances [97, 98, 51, 7, 5, 8, 7, 4, 94]

Until recently most investigators have stressed heparin or its precursors as the chief product of mast cell granules [500, 6, 6, 1094] However, this tenet does not necessarily indicate that the production and release of heparin is the only, or even the chief, function of mast cells [99, 5, 1, 5, 8, 693] Indeed Michels[693] (1938) states that at least 25 hypothetical functions of mast cells had been enumerated

Circulatory Relations

Mast cells store heparin, which it is claimed is loosely bound with histamine, probably within the mast granules,[6, 7, 855, 108] or on the surface *of the granule* [83] *The presence of numerous mast cells in a structure or area,* thereby assures an available supply of heparin at that locus This assumption is supported by the numerous observations that there are practically no mast cells or histamine in the liver kidney spleen or brain of the rat [8, 5] but that there is a correlation between the presence of mast cells histamine and heparin in the liver of cattle [108]

Mast cells are numerous along small blood vessels or aggregated in the adventitia to form a "cuff" around the vessel Sundberg[99] made an exhaustive quantitative study of the mast cells in the wall of the aorta and great systemic veins in the trunk of a total of 145 autopsies of persons ranging in age from 24 hours to 88 years The average count of mast cells in the walls of veins of infants and school age children was 900 cells per cubic millimeter in men 20 to 39 years of age the count was 1200 whereas in women of the same age group it was 1600 and in old age the average was about 700 (for both sexes) per cubic millimeter of vascular wall The *walls of the aorta of the aged contained a considerably higher number of* mast cells than the walls of the vena cava [99]

A disturbing factor encountered in attempting to quantify the counts was that the mast cell count in all age groups varied from extremely high to low values especially in the preadolescent age groups *Al o 9 individuals* had a marked difference in counts between the vessels of the left and right sides A 72 year old man who died of meningitis and bronchopneumonia had an average count of 1756 for the right and 2242 for the left external iliac veins per cubic millimeter of venous wall whereas another man of 64 who died of peritonitis suppurative hepatitis and pneumonia had average counts of 118 for the right and 107 for the left external iliac venous walls [995] The e and a number of other cases given by that author indicate that multiple factors make it impractical to draw definite conclusions con

tamine from performing this function[1, 317, 3, 3] indicates that the absence of inhibition of heparin may play a very important part in the genesis of cholesterolopoiesis arterial atheroma and various related conditions Several investigators support this idea[508] Others point out that atherogenesis results from altered lipid metabolism rather than from senescence itself[98]

The discovery by Hahn (1943) that *intravenously injected heparin* abolishes alimentary lipemia in dogs[1131] led to the application of this procedure to clear blood and lymph of lipids The information obtained by *this line of investigation indicates that the effects of heparin injected in*travenously into rabbits and rats had parallel effects on the concentration of triglyceride and free fatty acid in blood and lymph[1131] Brown (1950) using *rats* and dogs showed that heparin rapidly cleared postprandial hyperlipemia and that the administration of protamine reversed the action of the heparin[317] That this clearing factor is activated by heparin, was demonstrated by the fact that injection of heparin cleared the lipemic sera and reduced or caused low density lipoproteins to disappear[73] Anfinsen[1] reports that the administration of anaphylactic agents produced high plasma levels of clearing factor in dogs presumably by releasing both heparin and histamine from mast cells It has been shown that antihistamines do not affect clearance of lipemia However Havers and Boyle have shown that the administration of small amounts of protamine subsequent to the prevention of shock by use of antihistamines depleted the plasma of clearing factor which was not affected by the antihistamines in dogs[1] Anfinsen[1] points out that the clearing reaction is a common process resulting in the formation of triglyceride hydrolyzing components in normal plasma and similarly in the lymph of rats and rabbits when injected intravenously or when postheparin rabbit lymph was added to lymph *in vitro*[1131] It has been shown that heparin injected intravenously in dogs probably clears alimentary lipemia by disrupting the globulin lipid bonds with the release of the combined lipids and the formation of a heparin *protein complex*[98] Anfinsen[1] *suggests that heparin may serve as a* component prosthetic group of clearing factor since it is required in the production of the clearing factor *in vivo* and *in vitro* He also suggests that mast cells are a source of heparin When antihistaminic agents were used to prevent the condition of shock following the administration of anaphylactogenic agents to dogs the plasma of these dogs contained high levels of the clearing factor which disappeared following the injection of protamine in small amounts[1]

The part played by heparin in fat clearance of lymph may be related to the significantly greater number of mast cells in the medullary cords of the pancreas Aselli (largest lymph nodes of the mesenteric series) than occurs in other lymph nodes in hamsters[535] A decrease in glyceride con

It has been shown that 2 0 mg of heparin, administered intraperitoneally daily to cholesterol fed rabbits suppressed the development of aortic atherosclerosis about 40 per cent, and prevented hepatic fat accumulation by about 60 per cent, as judged by gross and micro copic grading methods [680] Daily administration of heparin by Graham and co workers (1951) prevented the onset of arteriosclerosis in all but 3 rabbits of two groups that totaled 40 individuals which were fed cholesterol in the regular diet [995] Fifteen of the control rabbits which had the same cholesterol diet but received no heparin, developed atheromatous foci [995] The fact that rabbits, which have relatively few mast cells,[693] are susceptible to cholesterol induced arteriosclerosis[995], and rats which are richly supplied with mast cells,[693 855] are not susceptible supports the contention that mast cells supply readily available heparin which functions in clearing the blood of excess lipids and cholesterol

A clinical approach to this problem was employed by Cains and Constantinides (1954), who found a much lower number of mast cells per unit of cardiac muscle in 48 people 60 to 90 years old who had marked arteriosclerosis than in the same number and age group who did not have macroscopically evident arteriosclerosis [99] There was no sex difference in the number of mast cells in these two senescent groups, but in 46 individuals 20 to 35 years old who had marked arteriosclerosis the males had a lower mast cell count than the females [995] Hueper, in 1941 to 1942, suggested that the instability of the colloidal solution in hypercholesteremia may start a sequence of events in the vascular wall and, by histiocytic phagocytosis of cholesterol which has penetrated the subendoethelial space, induce atheromatous lesions in arteries [508] Hirsch and Weinhouse (1943) consider that these two conditions represent the initial stage of atherosclerosis [508]

Hypercholesteremia may be induced by disturbance in lipid and/or cholesterol metabolism and/or elimination [508] Hypercholesteremia occurs under conditions of experimentally sustained hyperlipemia[508] (possibly neutral fat and/or phospholipid) in rats [3 3] Hypercholesteremia is to a considerable extent the result of cholesterol being retained by the excess plasma lipid [3 3] Friedman and Byers[3 3] found that continuous intravenous injection of soya lecithin and neutral fat was soon followed by hypercholesteremia in rats They also found that when pooled hypercholesteremic serum was exposed to a suspension of intestinal lymph lipids *in vitro* the seurm cholesterol content was reduced from 2 56 to 1 00 mg while the amount of cholesterol in the chylomicron lipids increased from 2 4 to 3 4 mg thus showing that the lipids had ' trapped more than 50 per cent of the cholesterol present in the hypercholesteremic serum Endogenous or exogenous heparin is a potent agent in clearing hyperlipemia and hypercholesteremia [1 3 3 508 73 879 933] The fact that protamine prevents his

lesterol esters[80] or as lipoprotein[3, 3, 3, 7] The information gleaned from the foregoing experiments seems to indicate that excessive plasma cholesterol arises through hyperlipemia and through the failure of its plasma fat clearing propensities

Summary of Activities

An incomplete summary of the activities of heparin shows that, in the presence of calcium and other essential substances, heparin strongly inhibits blood and probably other, coagulation that is prevents the release of histamine from blood cells and probably from other cells during anaphylactic and perhaps other forms of shock,[416] that it probably inhibits the liberation of histamine in various types of cell injury under normal conditions including radiation as well as necrotic and traumatic injury In addition heparin probably inhibits the release of histamine by trypsin or other proteolytic enzymes including proteolytic enzymes freed from injured cells Heparin suppresses mitosis by inhibiting DNAase and RNAase activity, reduces the lipid fatty and cholesterol content in hyperlipemia and hypercholesteremia and possibly plays a part in converting depot fat into glycogen

HYALURONIC ACID

HA is probably more widely distributed and plays a part in more physiological processes than is generally realized The source of HA is not definitely known[1] and the problem has been complicated by mistakenly identifying HA as mucin in the skin[1] synovial fluid[19, 68] and perhaps other structures By the use of chemical methods at least four investigators Chain and Duthie (1940) Claude (1940) and Meyer and Chaffee (1941) identified the so called dermal mucin in a variety of animals and Watson and Pearce (1947) in human skin as hyaluronic acid[1]

HA is a natural polymer of acetylglucosamine and glucuronic acid with a high viscosity and molecular weight of 200 000 to 500 000[19, 684] Acetyl glucosamine and glucuronic acid occur in equal parts in human umbilical cords[688] HA is a polybasic[1039] sulfate free carbohydrate[36] and combines with many proteins to form salts which precipitate under acid conditions[1039] HA is considered a polymeric electrolyte[37] which is thought to occur in the body as a salt with inorganic bases[684] Results of other work indicate that HA is derived chiefly if not entirely as a polymer of the disaccharide N acetylhyalobiuronic acid[697]

However with toluidine blue staining metachromasia of connective tissue does not clearly differentiate hyaluronate from sulfate MPS[698] Meyer[691] believes that HA is important in the composition of mesodermal ground substance but cautions that chemically only the structure of

tent and an increase in free fatty acids in the intestinal lymph of rats and rabbits follows the intravenous injection of heparin [11 1] The fact that this reaction was most marked in the intestinal lymph, which had a higher relative amount of triglycerides than either the hepatic or thoracic lymph, indicates that heparin has a fat clearing capacity Addition of lymph from rabbits previously treated with heparin *also produced lipolysis of a fatty* substrate *in vitro* [1131]

The action of heparin in clearing lipemia is generally accepted, but the explanation of its mode of action has not been established A number of investigators have observed that there is an increased concentration of free fatty acids in the plasma of man or experimental mammals after the intravenous injection of heparin, and that fatty acids are formed when heparin is added to a fatty substrate *in vitro* They attribute the produc tion *of these free fatty acids partly, probably chiefly, to glyceryl ester* hydrolysis [93 1131]

Weld (1944, 1945) found that heparin alone failed to clear lipemic serum *in vitro*, but that it was readily cleared by the addition of plasma from heparin treated individuals [1131] About 1950, Gofman and co workers con cluded that heparin in these *in vitro* experiments acted on the lipoprotein molecules [1131] Shore[93] believes that heparin catalyzed the release of fatty acids from lipoprotein triglycerides by activating a triglyceride splitting *enzyme He states that L thyroxine (THRX), which is the more potent*, and triiodothyronine (TRIT) inhibit this heparin catalyzed release of fatty acids from the lipoprotein triglycerides

Administration of heparin decreased experimentally induced hypercho lesteremia that was accompanied by hyperlipemia Rosenman and col laborators[879] found that heparin treatment decreased the hypercholester emia and hyperlipemia in rats having nephrosis induced by injecting rabbit anti rat kidney serum Friedman and Byers[3 3] show that prolonged intrave *nous injection of lipid mixtures of soya lecithin and neutral fat* quickly led to the appearance of hypercholesteremia which they attributed to 'some phase of fat metabolism They found that a suspension of chylomicronous lipids from intestinal lymph extracted over *half of the cholesterol origi* nally present in the clear hypercholesteremic serum ' *in vitro* Results of their various experiments led them to conclude that plasma cholesterol recovered by the liver was trapped by the excess plasma lipid

DeLalla and Gofman (1954) point out that in the normal serum of man and several other mammals most and possibly all of the cholesterol and phospholipids occur as lipoprotein [3 7] Others believe that normally, about 75 per cent of the plasma cholesterol is in the form of its esters [60]

Cholesterol *is generally considered to be an active metabolite* [60] Plasma cholesterol may occur as its esters of higher fatty acids [41] chiefly as cho

duction[21, 537] by the systemic alteration of connective tissue specifically, or by the alteration of the intercellular components [48] That the granules of mast cells may be a source of HA is not inconsistent with the fact that these granules fail to respond positively to tests for HA because the AMP in the granules could be changed for example by hydrolysis to glucuronic acid and glucosamine, and the latter, in turn, may form acetylglucosamine and polymerize with glucuronic acid [6] These processes could occur in the intercellular tissue fluid in the fibroblasts or in other cells of the connective tissue

Jeanloz (1950) states that polysaccharide in the synovial fluid is a polymer of D glucuronic acid and N acetylglucosamine [875] The mast cells which are abundant in connective tissue of the synovial membrane,[21, 96, 1107] could be a source of glucuronic acid glucosamine or sulfuric acid Acetylation of glucosamine and the subsequent polymerization with glucuronic acid might occur in any one or all four of the following sites (1) in the tissue fluid among the mast cells (2) in the tissue fluid between the mast cells and the synovial membrane (3) within the synovial membrane or (4) within the synovial fluid The combination of glucuronic acid and glucosamine seems to be more significant from the standpoint of granules of mast cells as a form of storage, than as a compound including these two substances

Meyer and co workers (1938 and 1939) state that HA in the body consists of equimolecular parts of glucuronic acid and N acetylglucosamine as a polymeric compound of a very high molecular weight around 200 000 to 500 000 [1, 684] They and other investigators point out that the jelly like character of HA in body fluids is related to the degree of its polymerization which is progressively reduced to a liquid state by depolymerization [8] A point of significance in this process is that permeability of structures or substances particularly the HA containing ground substance in connective tissue is related to the degree of depolymerization of the HA in it [6, 1, 56, 416] Schade (1923) believes that connective tissue fibers and ground substance possess important regulatory activity with regard to acid base water electrolyte and osmotic equilibria as well as tissue permeability [6] Thus by control of the polymerization of HA and other contained MPS the size and rate of passage of molecules through the group substance may be controlled to an almost unlimited extent [416, 87]

Relation to Ground Substance

The relation of mast cells to the formation of HA in ground substance is very difficult if not impossible to establish by histochemical methods because the ground substance contains both HA and AMP that contain the sulfate group Certain investigators believe that ground substance is com

the repeating unit, the disaccharide β glucuronido 1 3 *N* acetyl glucosamine" is known with certainty. Other investigators appear to support his statement,[60, 1004] but some believe that HA is sometimes classed as a MPS.[14, 60, 84, 107, 1089] Traut[107] lists HA and hyaluronosulfuric acid as two of the four MPS which occur in the cement substance of cartilage. Chemists[60] recognize mucoitin sulfate as the sulfuric acid ester of HA. HA is widely distributed in the human body, but it does not normally occur in the serum—its site of origin is not known.[17] The assumption that (1) the HA in connective tissue is produced by young fibroblasts, (2) HA in synovial fluid is produced by the synovial membrane, and (3) HA in the humors of the eye is produced by the ciliary epithelium, are merely presumptions based entirely upon probability.[71] Writers sometimes seem to use the terms hyaluronic acid, hyalurate and hyaluronate interchangeably.

Addition of electrolytes may greatly decrease the relative viscosity of the solutions.[37, 1089] For instance, a 0.15 per cent aqueous solution of sodium hyaluronate has a viscosity value of 9.45, whereas the same percentage solution made up in 0.1 N sodium chloride has a viscosity value of only 2.69.[694] Martin[648] suggests that ions probably play a significant part in enzymic depolymerization of HA and, consequently, in permeability changes. Another relevant point of interest is that different methods of isolating HA alter the viscosity. Nevertheless, its viscosity is almost never as high as that of the biological fluid from which it was isolated.[537]

HA in solution is an important lubricating agent.[60] Normal human synovial fluid is generally believed to have a high viscosity which is attributed to the presence of mucin,[8] but others attribute about 80 per cent of the viscosity and lubricating efficiency to hyaluronates.[60] This does not, however, preclude the possibility that synovial mast cells contribute to the dialyzate. Synovial fluid does not contain sulfate containing carbohydrates,[96, 87] although chondroitin sulfate is present in the ground substance from which this dialyzate is formed. This difference may indicate that sulfated MPS are more firmly bound to fibrous components of connective tissue.[875] The nature of synovial fluid may be related to the fact that "synovial tissue has a markedly greater permeability" than true membranes,[87] and a rich blood and lymph supply.[393, 107] These conditions appear to favor the passage of substances stored within the subsynovial connective tissue mast cells, which are abundant near the surface of the synovial membrane,[96] into the synovial fluid. The amorphous substrate of fibrous connective tissue is thought to contain HA which may exist either as gels or sols dependent upon the degree of polymerization.[60, 1089] However, the synovial fluid and the aqueous humor of the eye are always a sol.[746]

Some investigators involve the mast cell directly or indirectly in HA pro

reduction in mast cells is accompanied by a reduction in HA McManus[6..] has shown that the quantity of HA is correlated with the number of mast cells in the tissue Traut[10, 7] believes that synovial HA is produced chiefly by young fibroblasts Although mast cells are generally considered to be an important source of HA, certain investigators[181, 1107] point out that the metachromasia of the granules is not altered by treatment with hyaluronidase and that this treatment does not alter other staining reactions of mast granules[896] Asboe Hansen[19, 4] however reports that metachromasia was changed in the ground substance of skin biopsies subsequent to the injection of hyaluronidase

It is worth noting at this time that the relation of mast cells to the formation of HA in ground substance is difficult to establish satisfactorily by chemical means because the ground substance includes AMP that contain the sulfate group in addition to HA However Rice[843] was able to separate the sulfated from the non sulfated polysaccharides in an extract from rabbit syphilomas by using a modification of Pearce and Watson s (1949) method Fawcett[96] and others point out that mast cells are more frequent in connective tissues than is generally supposed because routine staining such as hematoxylin and eosin does not demonstrate these cells

Incidentally the records of counts of cells in synovial fluid are often disappointing because mast cells are rarely mentioned in either normal or pathological conditions When mast cells are not mentioned there remains the possibility of dissolution of the mast granules in the diluting solution rather than the actual absence of these cells Ropes and Bauer[9] state that it was necessary to dilute the fluid in a white cell counting pipette with normal saline instead of routine diluents to avoid precipitation of the mucin This may be a special case in which the investigators planned to destroy the mast cells in order to favor counting other cells just as erythrocytes are destroyed with acetic acid to favor obtaining a differential leukocyte count We[6..3] find that granules of mast cells in tissues fixed in sublimate alcohol do not dissolve when deparaffinized sections are placed in water or salt solution However the exposure of tissues that contain mast cells to most aqueous solutions causes the granules to dissolve[99, 893] and prevents identification of the mast cells

MUCIN IN SYNOVIA

In his extensive review of the literature on the chemistry of mast cell granules Michels[6, 3] states that Roundnitz (1883) believed that these granules are a mucolecithid compound and that Harris (1910) contended that mast granules are composed of mucin which is elaborated as the mucin associated with connective tissues Michels reviews the historical background of the works of a number of individuals and states that Kultschitzky

posed chiefly of HA, derived in major part, if not entirely, from mast cells [1, 4] Asboe-Hansen (1951) suggests that the function of mast cells is to produce connective tissue ground substance, but Burkel (1952) believes that mast cells do not produce HA [1091] Compton[181] was unable to identify HA in granules of mast cells of hamsters This failure would be expected in the light of the known storage functions of mast cells, because one would not expect to find HA stored in a free or active state within the mast cells However, Klemperer[548] suggests the possibility that instead of being produced by "fibroblasts or other cells (mast cells)," the components of normal ground substance may be "directly transferred from the blood plasma" just as the abnormal intercellular component, paramyloid, is deposited in connective tissue

HA occurs throughout the connective tissue ground substance [1, 4, 6, 7] where it is believed to have been derived from a prestage condition in mast cell granules [71, 4] Since mast cells are nearly always present under these conditions, if this relationship is widely established, potential HA would reasonably be expected to occur wherever mast cells or mast cell derived AMP are found However objections based apparently on chemical incongruity, have been raised against the belief that the amorphous ground substance is supplied by mast cells [6] Burkel (1952), believes that instead of HA being present in mast granules in a prestage, it is more likely that HA is a prestage of heparin [99] Altshuler and Angevine[6] suggest that the intracellular AMP of mast cells may very well be produced by certain influences which encourage production of AMP in the ground substance

HA inhibits the activity of phosphoacylase Thus, when it is in excess as a result of the activity of two known sera or other hyaluronidase inhibiting substances,[1104] HA may inhibit hydrolysis of acetyl phosphate to form phosphoric and acetic acids in muscle as well as in the brain and kidney [993] It is not known just how, or whether, this reaction may be related to HA in the synovial fluid

Mast Cells and Synovial Fluid

Whether or not HA that is derived from mast cells is present in a depolymerized inactive or other state in the dialyzate that forms the synovial fluid, is controversial Normal human synovial fluid (synovia) is generally thought to be a dialyzate of blood plasma rather than a secretion [176, 96, 393, 87, 107] although it is considered by some to be both [107] and by others to be secreted chiefly by certain synovial cells [107] Whether mast cell [1, 4] and/or fibroblasts[107] supply the material for its viscosity is another debated point on the origin of synovia

Mast cells are believed by a number of investigators to be an important source of hyaluronic acid [18, 70, 4, 1, 0, 6, 996] Cavallero[150] observed that a

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(1897) was the first to show by various staining procedures that mast granules are not identical with the substance contained in goblet cells. One gets the impression that a number of investigators feel that the mucin (probably HA) in synovial fluid may be derived, at least in part, from mast cell granules. There is evidence that mucin may be derived from mast granules in other situations.[71, 7-8] Nevertheless, few investigators are willing to take the stand that mast cells are a source of either the mucin or the HA found in synovial fluid.

It is generally conceded that the concentration of synovial mucin in joint diseases is related to the type, severity and duration of the disease and the duration of the effusion. Production of both synovial fluid and mucin is greatly increased in traumatic injury, but the ratio of mucin to fluid remains normal.[8] Bennett[63] and Ropes and Bauer[875] suggest that the destruction of normal cartilage may contribute to the high mucin concentration in the synovial fluid in degenerative joint disease. Since a myxedematous patient was found to have an abnormally high concentration of synovial mucin, it is suggested that thyroid secretion plays an important part in the metabolism of mucin in both the synovial fluid and in the subcutaneous tissues by either accelerating the production or by retarding the degradation of the mucin.[87] The finding of an abnormally high concentration of mucin in the synovia in this myxedematous patient is interpreted as an indication that hypothyroidism changes the formation of mucigen.[8] Preliminary work with hamsters into whom estrogens were injected subpannicularly[198] *indicates that this hormone acts upon the mast cells to free* the granules, rather than to inhibit mucin metabolism in the animal.[533]

Mucin, which is "the characteristic component of synovial fluid and which is responsible for the high viscosity of the normal fluid," is thought to be a product of periarticular connective tissue cells. Therefore, it has been suggested that the mucin is present in the tissue fluid before it enters the synovial fluid.[8] However, Traut[10] believes that the mucin is secreted by specific cells in the synovial membrane. Various investigators show that normal human synovial fluid contains from 0.068 to a possible 5.7, with an average in one series of 0.104 per cent of mucin determined as nitrogen, with an average of 0.087 per cent of mucin glucosamine,[8, 5] or as varying from 0.3 to 0.8 gm/100 ml of synovial fluid.[2, 8]

Ropes and Bauer[875] counted the white blood cells and determined the amount of synovin (synovial mucin) of patients that had several pathological conditions. They concluded that there is a great increase in the amount of mucin in the synovial fluid from degenerative joints and especially from Charcot joints (up to 0.208 per cent) and from synovial osteochondromatosis (average 0.166 per cent). Ropes and Bauer[875] also attribute the increase in synovin to an increase in the formation of mucin as a response to the

stimulation of the tissues which was not accompanied by the increase in destruction of synovial mucin, characteristic of highly inflamed joints Bennett [69] however points out that these conditions also occur in the formation of collagen or of cartilage

It may be concluded from the above statements and tenets that mast cells probably play an important part in the formation of synovial fluid, whether its viscosity is due to the presence of HA or of mucin or whether synovia is a dialyzate or a secretion

HYALURONIC ACID IN SYNOVIA

HA is said to be the only form of AMP normally present in synovial fluid [9] However some writers use the term mucin to include HA [1 6] whereas others[875] recognize the presence of both HA or its salts and AMP The highest concentrations of HA in the normal mammalian body are found in the umbilical cord skin and synovial fluid [1099] About 80 per cent of the viscosity of normal synovial fluid may be attributed to its content of 0 02 to 0 5 per cent of hyaluronate [69] normal human synovial fluid has been reported to contain concentrations of HA that ranged from 0 04 to 0 33 mg/ml [875]

The source of synovial HA has not been conclusively established but there is the probability that a significant amount of it or of its components or salts is derived from extrasynovial mast cell granules and/or fibroblasts Young fibroblasts have been indicated as its source [10 7 1099] Asboe Hansen [4] believes that at least in the skin fibroblasts play a part in fibrilogenesis but that mast cells secrete important components of the mucinous ground substance primarily the viscous mucopolysaccharide hyaluronic acid

Enzymic Activity

The number of unknown enzymes or factors coenzymes and cofactors and the possible combinations of these which directly or indirectly affect HA and other MPS for that matter is probably much greater than is generally appreciated In addition ionic and hormonal changes[1059] and pathological conditions play an important part in altering the normal activities of enzymes as well as the activities of the enzyme inhibitors Enzymic degradation of HA appears to be a change undergone by this substance in arthropathy [6 4 10 7] Also the hyaluronate in the synovial fluid is depolymerized in tubercular arthritis Whistler and Smart[1089] state that there is evidence that rheumatoid arthritis may be due to a biosynthetic deficiency in or failure to complete polymerization of hyaluronate and that the administration of D glucuronic acid has been found to have a beneficial effect in some cases This tenet is supported by the recent finding that the relief

brought to some arthritic patients by oral administration of D glucuronic acid "may indicate that the added uronic acid assists in the production of complete hyaluronic acid molecules"[1089] The fact that cancer, poisons and pregnancy all raise the polysaccharide level in the blood serum significantly,[1089] suggests that part of the beneficial effect of pregnancy on arthritis may be due to an increase in the level of serum polysaccharide which, in turn, could increase the availability of D glucuronic acid for proper formation of HA acid in synovial fluid

Uses of the terms "Duran Reynals factor," "spreading factor," etc, as synonyms of hyaluronidase have been deplored Meyer[686] suggests that the term "spreading reaction" should replace the use of the term "spreading factor"[7] Hyaluronidases are separated into two groups (1) those of animal and (2) those of microbial origin Enzymes of the two groups differ somewhat in mode of action and in affinity for the substrate Several hyaluronidases act upon HA hyaluronate or other MPS Meyer and Rapport (1952) found that all of the hyaluronidases that they investigated attack native polymers which have either high or low molecular weights by opening the glucosaminic bonds but those which act upon HA attack only the glucosamidic bond of the two glucosidic bonds occurring in this MPS[6,7] Hyaluronidase depolymerizes and hydrolyzes HA[39] by liberating the disaccharides Any further degradation is due to other enzymes"[6,1089] Several investigators have shown that the jelly like character of HA or its salts in solution and its consequent ability to inhibit movement of fluid substances within the tissues is dependent upon the degree and asymmetry of polymerization[6] Hyaluronidase destroys the viscosity of the jelly like HA by degradation of the polysaccharides[6] and presumably of other MPS Thus hyaluronidase greatly increases the permeability of the tissues and tissue fluid[6,6,79,416,993,1051,1084,1089] Duran Reynals[6] crystalizes the application of the idea of the spreading reaction by stating that polymerized complexes of polysaccharides in ground substance are normally in the form of a gel and as such, they inhibit or form a barrier or obstacle to the absorption of injected substances such as a vaccine virus but if hyaluronidase is added to the substance before it is injected the substance readily spreads through the tissue as does a fluid dropped upon a blotter He points out that this change in rate of absorption is the result of conversion of the gel to a sol by the degradation of the polysaccharides Also electrolytes are probably involved[648] because others have shown that hyaluronidase activity increased the concentration of sodium and potassium in synovial fluid of dogs[1089]

Two or more hyaluronidase inhibiting substances which differ in heat lability, in levels of activity in pathological conditions in specificity for hyaluronidases obtained from different sources and probably in other ways,

have been found in serum Glick and collaborators (1948 1951) found that certain mast cell rich tissues are probably the sites of origin of one of these hyaluronidase inhibitors which may be a heparin lipoprotein complex and which occurs in an albumin fraction as a constituent of normal plasma but shows no relationship with plasma mucoprotein [1104]

Cortisone which has a marked palliative effect in some cases of arthritis has been reported to act by inhibiting hyaluronidase [1059] That this effect of cortisone pertains only to the action of hyaluronidase upon HA is supported by Humphrey (1946) who found that hyaluronidase has no action on heparins or mucoitin sulfate [1059] This difference suggests that cortisone does not act directly upon hyaluronidase but that it acts directly upon some biogenetic agent which is essential for the production of this enzyme as has been shown for its action in inhibiting histidine decarboxylase in the synthesis of histamine [392] Mathews and Dorfman[6, 4] present another angle by warning that the belief that hyaluronidase is etiologically related to rheumatic disease has no evidence to support it They suggest that other enzymes may be affected by some of the inhibitors of hyaluronidase A number of investigators have shown that the administration of cortisone hydrocortisone or adrenocorticotropic hormone produces favorable clinical effects on a large percentage of patients suffering from rheumatoid arthritis Some of these authors attribute the beneficial results to the inhibition of hyaluronidase or some other enzyme action on an unknown polysaccharide presumably HA It has been shown that heparin can inhibit hyaluronidase *in vitro* [47] One group reports that they believe that the treatment of these patients enabled them to make a longer chain polysaccharide [166] but another group suggests that the remissions were due to an alteration of an enzyme chain [440]

If tissues were treated with hyaluronidase the effect presumably would be limited to the liberation of the disaccharide components of the HA [6] [1059] However preliminary study of slides treated with hyaluronidase has not been successful because the hyaluronidase was used in physiological saline solution which had a pronounced affect in altering the metachromatic staining of the mast cells The exposure of AMP to water also altered the density and degree of taining of white fibrous connective tissue by Mallory s triple method In order to evaluate the true effect of hyaluronidase it is nece sary to avoid exposing the tissues to sodium chloride or other water solutions because the changes produced by these substances alter all AMP and would prevent obtaining a true effect of the enzyme on HA

8

The Thymus

The thymus differs from other lymphoid tissues in being more sensitive to agents that deplete lymphoid tissues and in involuting at puberty Absence of afferent lymphatics is the main anatomical difference between the thymus and lymph nodes All lymphoid tissues except the thymus and that in the mucosa of the intestine contain afferent lymphatics and have large medullary lymph sinuses The sparsity of lymph sinuses in the thymus (fig 16) may be a part of the basis for the greater sensitivity of the thymus to stimuli which cause involution of lymphoid tissues There is less dilution by tissue fluid and lymph of a hormone such as cortisone and other lymphopenic agents that are carried in the blood to the thymus, than occurs in other lymphoid tissues

The thymus is composed primarily of lymphocytes which as they develop, synthesize and store deoxyribonucleic acid (DNA) and the amino acids present in thymus histones This arrangement enables the thymus to play a highly specialized although very significant part in nucleoprotein metabolism However the thymus has this function primarily during childhood because it begins to involute at puberty as protein synthesis increases in the gonads The inverse relation of changes in the size of the thymus to gonadal function resulted in the idea that the thymus is an endocrine gland However, Hammar,[398] as early as 1910, realized that the thymus does not have a secretory function and Hoskins (1918) agreed with this opinion[393] In 1920 Dustin stated that the thymus does not function by means of secretion" and in this way it is unlike organs of internal secretion Nevertheless for many years experiments were designed to demonstrate that Hassall s corpuscles have an endocrine function The idea that the thymus is an endocrine gland still persists[493, 1136] with the result that some writers are unable to decide whether to classify the thymus as a lymphoid structure or as an endocrine gland and compromise by referring to it as the ' thymus gland "

As early as 1904 Bang reported five times as much nucleic acid in thymic tissue as in lymph nodes[638] Cooper (1832) suggested that the ' thymus gland ' probably is designed to prepare a fluid that is well fitted for fetal growth and nourishment from the blood of the mother before the birth of the fetus[638] Later Simon concluded that the thymus functions as a sink

ing fund' of nourishment which is produced as a fluid secreted in early life[638] Simon also noted the sensitivity of the thymus to malnutrition and accordingly designated this structure as a 'barometer of nutrition'[471] Dustin[60] considered thymocytes as regulators of nucleoproteins, and Klose in 1910 after learning that Bang in 1904 had shown by chemical analysis that thymic tissue contains at least 5 times as much nucleic acid as lymph nodes expressed the opinion 'that furnishing nucleic acid to the organism could be an important function of the thymus[638]

Reluctance to recognize the thymus as a lymphoid organ has been partly due to its differences in structure and embryonic origin from other lymphoid tissues The thymic parenchyma develops from epithelium, chiefly from the entoderm of the third and fourth branchial clefts in most animals[493, 638] instead of the mesoderm from which the lymph nodes arise[600] The thymus does not have afferent lymphatics[435, 638, 9, 6] typical lymph sinuses[141, 435, 493] or true germinal centers[141, 493] as do lymph nodes but the thymus is probably more vascular[493, 85] The possibility of a difference in embryonic origin[638] led to the belief that thymocytes are not the same as lymphocytes Marine[638] points out the obvious fact that the available information is insufficient to establish this identity although lymphocytes and thymocytes resemble each other in (1) ameboid movement (2) morphologic characteristics (3) serological reactions (4) susceptibility to x ray injury and (5) general pathologic reactions Jordan[493] believed that thymocytes are lymphocytes Now the thymocytes are considered genuine lymphocytes[44] and capable of becoming plasmacytes[1030] instead of being analogous to lymphocytes as Hammar (1905) believed[51] The thymus is now recognized as an important source of blood lymphocytes[638]

THYMIC INVOLUTION

Accidental involution of the thymus occurs rapidly and at any age Normal involution of the thymus begins at the onset of puberty and increases with age[638] Most agents or conditions which induce accidental involution of the thymus cause involution of the other lymphoid structures if continued Hammar (1905) states that the massive migration and destruction of the small thymic cells are brought about in some unknown way during accidental involution with the result that the organ may shrink to one fifth of its previous weight within a week The relative destruction of the medulla and cortex varies somewhat with the different kinds of accidental involution[638]

The thymus is generally conceded to be more sensitive to lymphopenic factors than the other lymphoid structures[9, 4, 61] However certain agents such as some arsenical preparations are reported to deplete the systemic lymphoid tissue first[84] Kirshbaum and associates[43] report that

thymic tissue apparently is more sensitive than nodal tissue This was especially noticeable in mice treated with both x rays and methylcholanthrene which acted synergistically

Thymic involution has been produced in a variety of ways Dustin and co workers (1913 to 1933) produced lymphoid involution in frogs and mammals by the use of a wide range of agents which they called "caryoclastic poisons,"[64] *e g*, dyes chemicals, x rays and toxic sera Dustin[61] and Dustin and Gregoire-[64] show that certain agents which deplete the thymus have selective effects on the cells of different lymphoid structures and other tissues Some agents appear to act directly upon the lymphocytes in the lymphoid tissue by causing pycnosis in a large percentage of the cells *Others such as vitamin deficiencies and hunger, appear to act indirectly by* producing inanition

One obvious cause of thymic involution is the release of adrenocortical steroids Thymic involution has been produced in normal animals by (1) the administration of adrenocorticotropic hormone ACTH,[91, 704] (2) hypophysectomy in rats[91] and (3) the administration of cortisone in rats, mice[8, 135, 46, 463, 9, 1, 939, 971] and hamsters[06, 0]

The significance of the sudden dissolution of relatively great numbers of thymocytes in accidental involution has not been determined The assumption that the thymus synthesizes and provides a readily available source for many essential substances is one possible explanation of the response known as accidental involution Thereby, the sudden withdrawal of lymphocytes from the thymus would provide other cells with histones, nucleic acids and possibly certain other substances Dustin[6] (1931) thought that the diverse causes of rapid thymic atrophy such as sickness inanition suppuration and formation of sex products,[60] were a response to the need of the organism for a great amount of nucleoprotein

VASCULARITY OF THE THYMUS

It appears that the unusual vascular architecture of the thy*mus affords* a logical basis for a morphophysiological interpretation of the extreme sensitivity of this lymphoid structure to lymphopenic agents[535]

The thymus of man is supplied by four to six blood vessels which enter and leave in different areas The arteries arise from the internal mammary thyroid[450, 638, 660] and pericardial arteries[618] The larger thymic arteries pass along the interlobular connective tissue and send branches to penetrate and supply the lobules These form a plexus of sinusoidal capillaries with elongated meshes in the medulla whereas the cortex of the lobules is supplied by radiating capillaries[493] The thymic arteries in the rabbit as in man are multiple and do not enter at a hilus The largest of them arises from the sternal artery[53] Smith and associates[95] describe five vascular

patterns of the mouse thymus. The thymic veins arise as sinusoidal capillaries[493] in the medullary regions and empty into the left innominate and thyroid veins in man.[430, 660] The thymic veins are thin walled, capable of enormous dilation and thereby are able to impound increased amounts of plasma proteins to facilitate lymphopoiesis and to prolong the action of retained substances which lyze the lymphocytes.

The thymus is the only lymphoid tissue, except that in the mucosa of the intestines, which does not contain afferent lymphatics and lymph sinuses. However, intestinal lymphoid tissue has an increased amount of tissue fluid from the absorption of liquids from the lumen of the intestine. Lymphatics of the thymus were first described in 1655 by Bartholinus.[9, 6] Warthonus (1659) believed that these lymph vessels should end directly in the subclavian vein without the intervention of lymph nodes, and Drelincourt held this view for the lymphatics of the thymus in the dog.[9, 6] Afanassiew[9, 6] (1877), His[435] (1861), Matsunaga[638] (1910), and Marine[638] (1932) thought that the lymphatics in the thymus arise from tissue fluid within the individual lobule and form plexuses of true capillaries in or on the periphery of the individual follicles, then form collecting interfollicular and interlobular lymphatics which pass from the interlobular connective tissue areas of the thymus in three directions toward its periphery to form the superior, ventral and dorsal groups of lymphatics.[9, 6] These thymic lymphatics drain the thymus and constitute the afferent branches of three sets of lymph nodes in man: the superior nodes (two), the anterior or ventral anterior mediastinal nodes (four to seven), and the dorsal nodes (two on either side).[9, 6] The efferent lymphatics of these three sets of thymic nodes empty into the subclavian vein, the cervical trunk, and/or the subclavian lymphatic trunk.[9, 6]

Functionally, the cortex of the thymus forms an extranodal cortex for the thymic lymph nodes, since the afferent lymphatics arise in the cortex of the thymus. Correlated with this lymphatic arrangement is the presence of an inordinately large number of plasmacytes in the medullary region of these thymic lymph nodes.[63]

Lymph sinuses are very rare in the thymus. Therefore, there is less stasis of lymph and less exchange with tissue fluid than occurs in lymph nodes. *Conditions are more favorable for the uptake of intercellular substances by the developing thymocytes.*

Vascular differences and consequent changes in the intercellular fluid may suggest a basis for determining the chief functional differences between the thymus and the other lymphoid tissues. It is suggested[33] that the absence of afferent lymphatics and the consequent absence of afferent lymph and lymph sinuses is favorable for the condensation of the substances which have passed from the very numerous sinusoidal blood capillaries into

the intercellular fluid of the thymus The chief cause of the greater sensitivity of the thymus to lymphopenic agents may be lesser dilution by lymph and tissue fluid

DEPLETION OF THYMUS

Depletion of the thymus is due to the decreased formation of lymphocytes or to increased disintegration of these cells Nutritional deficiencies which interfere with the synthesis of nucleic acids and histones deplete the thymus and other lymphoid structures The effect of administered ACTH or cortisone in depleting the thymus and other lymphoid tissues is striking and consistent There has been a tendency in the last 10 years to attribute the depletion of lymphoid tissue to endogenous adrenocortical steroids through stress reactions Thymic involution in tumor bearing rats has been attributed to a hyperactive adrenal or to involution caused possibly by a pituitary factor which has not been demonstrated in crude pituitary extract [58] However, this involution of the thymus in these animals could be a result of inanition especially if the rats had reached the state of carcinemia Starvation, malnutrition, vitamin deficiency or low protein diets decrease the formation or utilization of DNA and amino acids These conditions also deplete the thymus as well as all other lymphoid tissues [535] Inanition is a potent agent in producing thymic involution Thus, it interferes in evaluating the results of experiments designed to demonstrate the effects of a single dietary deficiency on lymphoid tissue

The thymus which Simons designated as the "barometer of nutrition,"[471] is depleted by several types of dietary deficiencies [471] The extent of depletion of the thymus varies with the duration of inanition—from a 75 per cent weight loss from acute inanition, to a 90 to 100 per cent loss in chronic inanition [471] The depletion of the thymus by starvation was observed by Jonson[367] (1909) Jackson[9] (1915) Stewart[9] (1916) Jolly [51] (1914) and Jolly and Saragea [51] (1924) The subject is reviewed by Drinker and Yoffy [51] Andreasen[9] and Marine [638] Hibernation also depletes the thymus in hedgehogs [898] frogs [63 445] and probably other animals Marasmus and chronic illness deplete the thymus in men [141 60 713]

The extreme involution caused by starvation suggested that the thymus plays a significant part in normal nutrition[638 1007 1136] and that lymphocytes in the thymus contain "substances of importance to the growth of the individual "[9] Andreasen[9] and Jackson[471] evaluate the work of many investigators and show quite conclusively that hunger or inanition, in proportion to its severity and duration may profoundly deplete all lymphoid tissue in animals Many of these investigators report that the thymus is more severely depleted than any other lymphoid structure, especially in adult individuals Jackson (1915) and Stewart (1916) found that chronic malnutrition resulted in marked weight loss by the thymus [9] Jolly (1914)

using prepuberal animals, found that after 6 to 7 days without food the thymus of the dogs lost 68 1 per cent and that of the rabbits lost 87 9 per cent in weight [9] The thymus in a group of 22 3 month old male and female rats without food for 7 days lost an average of 91 0 per cent of its normal weight, whereas the loss *in fatty acids was 92 0 per cent of the normal* weight [9] Andreasen[9] also found that when these starved young rats were realimenated, restitution of the thymus was much slower than the body The thymus had regained only 50 per cent of its normal weight and the body weight was 95 per cent of normal on the 8th day of realimentation

Chronic inanition depletes all lymphoid tissues [51 471 489 490 518 53 547] Andreasen[9] found that the thymus of rats is initially more sensitive and that it loses weight faster than the nodes in progressive depletion, and the nodes lose weight faster than the spleen Accidental involution of the thymus occurs in children and adults Wasting illness causes a more rapid atrophy of the thymus than of other lymphoid tissues [715] However atrophy *of the spleen and Peyer s patches occurs coincidently with atrophied thymi* in children dying in a state of malnutrition and marasmus [14]

Involution of the thymus accompanying illness may be due to any one or a combination of several conditions [1136] Decreased intake and assimilation of protein and B vitamins or increased protein catabolism are common causes [71] The condition of status lymphaticus is now believed to have been erroneously [1136] thought to explain the sudden deaths in which thymic hyperplasia was the only clue to the cause of the sudden death The condition was consequently diagnosed as acute thymic hypertrophy [1136] and was explained by Rokitansky as having caused death by mechanical means associated with enlargement of the thymus or to an unknown toxic factor [1136] Paltauf associated the thymic hypertrophy with narrow aorta cardiovascular hypoplasia and lymphatism and suggested that there was an interrelationship between the thymus and the adrenal glands [1136] *More recently pathologists have attributed the cause of death in cases* of status lymphaticus to other causes such as anaphylaxis [1136] They believe that the size of the thymus is indicative of the normal condition in contrast to the smaller size of the thymus observed in autopsy following prolonged illness

DIETARY DEFICIENCIES

Deficiency of amino acids or of single or multiple fractions of the B complex depletes the thymus [14 2 1 667] Although deficiencies of the B vitamins usually have systemic effects on reducing lymphoid tissues [21-] infiltration of lymphocytes still may occur in some lesions *e g* the cornea in ariboflavinosis [713 80] the dermis underlying the thickened epidermis in pellagra [1069] and in the medulla of enlarged adrenals [67]

A number of papers that discuss the effects of amino acid deficiencies on

the thymus have appeared in recent years Deficiency of different essential amino acids depletes the thymus and other lymphoid tissues of lymphocytes Young male rats fed a synthetic diet that lacked only threonine, one of the essential amino acids in thymic histone, had the thymus excessively depleted compared with those of "starved control rats," and also had other deficiency effects which disappeared when the missing amino acid was added to the diet[91] Leucine deficiency also caused atrophy and other changes of the thymus[1094] Deficiency of phenylalanine has been proved to cause atrophy of the thymus[914] This effect of phenylalanine is significant because it is an essential amino acid that is not usually identified as a component of thymus histone

Deficiency of various fractions of the B complex which have produced atrophy of the thymus in experimental animals include thiamine,[743] riboflavin, pantothenic acid,[995] folic acid,[431] pyridoxine,[844, 985] pteryolglutamic acid (PGA),[115] and choline[265] The cause of the depletion of the thymus by the absence of the B vitamins is attributed to inanition[51] and loss of body weight[985] For example Butler and Morgan[1 8] found a significant reduction in the weight of the thymus accompanied by lymphopenia, in pyridoxine deficient rats They hesitated to attribute the loss in thymic weight and circulating lymphocytes to pyridoxine deficiency because similar changes follow inanition

Dietary deficiency is a potent factor in thymic involution This is shown by the fact that avitaminosis of thiamine, riboflavin and pyridoxine causes (1) progressive weight loss, (2) progressive atrophic changes in the wall of the gastrointestine (with mucosal hemorrhage), (3) ulceration and (4) the destruction of ganglionic cells in the plexi of Meissner and Aurbach in hamsters[743] Pyridoxine deficiency causes the development of lesions in regions of the lymphoid follicles and atrophy of columnar epithelial cells[97] Niacin deficiency interferes with the functioning of the Golgi apparatus,[488] and causes mucosal lesions in the gastroenteron of man[80] Pellagra which results from multiple vitamin B and other deficiencies[78] is accompanied by lesions in the gastrointestinal mucosa[80] and liver[344]

It is not surprising that a number of earlier investigators believed that "an abundant supply of the water soluble B vitamin stimulates the functional activity of the lymphoid tissue and increases the number of lymphocytes in the circulating blood'[14] They also believed that withholding B vitamins disrupts functional activity of the lymphoid tissue similarly to that produced by exposure to x irradiation[14, 15]

The favorable effects of supplements of B_{12} on the atrophic thymus of the chick apparently is related to its PGA content because B_1 had no effect in the absence of PGA[115] Dietary deficiency of PGA is credited with causing atrophy of the thymus lymphopenia and several other disorders in chickens[889] Aminopterin (alone) an analogue of folic acid, produced folic

acid deficiency with atrophy of the thymus spleen and bone marrow—and leukopenia when given to normal animals However the e deficiency symptoms did not appear when folic acid was administered with the analogue [431] These results indicate that "for a certain period a given amount of vitamin will completely nullify a given amount of antagonist" [431] This complexity is illustrated by the observation of Meites (1951) that vitamin B_{12} and Aureomycin completely prevented thymic atrophy in young rats kept on cortisone and a diet deficient in B_{12} for 30 days [464]

Avitaminosis of certain B vitamins depletes lymphoid tissue and causes lymphopenia However it is difficult to determine whether one or more fractions of the vitamin B complex was actually the causative agent Inanition and the absence of certain other vitamins or fatty acids [97] secondary infections [51] and a number of other agents or conditions cause lymphopenia and depletion of the thymus and other lymphoid tissue Other sources of confusion lie in the variable results reported by earlier investigators who did not take into consideration the ability of some of the laboratory animals to supply certain vitamins by intestinal biosynthesis or coprophagy

Apparently deficiency of any of the B vitamins causes anorexia and interferes with digestion absorption and internal synthesis of proteins as indicated by the presence of lesions of the gastrointestine and liver [78] Absence of thiamine riboflavin or pyridoxine from an otherwise balanced diet reduced secretion by salivary and exorbital lacrimal (pouch) glands [116] and caused spasticity and ballooning accompanied by atrophic changes in the ganglionic cells mucosa and muscular layer of the intestine [743] in Syrian hamsters Thus it is not surprising that the absence of various vitamin B fractions may interfere with the digestion and absorption of other vitamins amino acids and components which are essential for the synthesis of nucleic acids Deficiency of B vitamins also decreases (1) the formation of certain coenzymes and (2) the synthesis of proteins and nucleic acids in lymphoid and other tissues The effects on the lymphoid tissues are more obvious because the cells of these tissues lymphocytes are storage cells for nucleoproteins

B_{12} the cobalt vitamin has a special function in hematopoiesis [141] in addition to increasing appetite and having functions in nucleic acid and protein metabolism It is a potent erythropoietic agent [389] and is an effective antipernicious anemia substance [368, 501, 1048]

SYNTHESIS OF NUCLEIC ACID

Many of the B vitamins have functions in the synthesis of nucleoproteins and provide components for the synthesis of the nucleotides Indirectly they are sources of components of coenzymes which are important in the synthesis of nucleic acids For example folic acid together with B_{12}

thymidine, and the citrovorum factor, function in the synthesis of nucleosides from parent pyrimidines and purines [78, 161]

One pyrimidine precursor is orotic acid which occurs in milk,[3, 4, 388] and appears to be the growth factor described by Novak, Hauge and Carrick in distillers' dried products [636] Orotic acid is similar to vitamin B_{12} in absorption spectra [636] The importance of orotic acid as a pyrimidine precursor in mammals cannot be accurately evaluated because there are other sources and means by which pyrimidines are synthesized For example, aspartic acid has been found to be a precursor of orotic acid and, therefore, of pyrimidine pentose nucleic acids [835]

The functions of vitamin B_{12} in nucleic acid metabolism are difficult to determine because of various interrelations of B_{12} with the formation of other vitamins Vitamin B_{12} is thought to play an important part in the perparation of carotine for conversion to vitamin A [43] Also, vitamin B_{12} stimulates the synthesis of folic acid, which in turn stimulates synthesis of additional vitamin B_{12} [34] Numerous functions which have been ascribed to B_{12} in nitrogen metabolism include increasing the incorporation of circulating amino acids into tissues[155] and playing a part in the synthesis of ribose, as is indicated by the finding that red cells from B_{12} deficient rats formed less ribose than did erythrocytes from controls [610] Vitamin B_{12} appears to function with folic acid in the formation of thymidine, the desoxyriboside of thymine, because thymidine can replace vitamin B_{12} in certain microbiological activities thymine can replace folic acid but not B_{12} [542] Another reason that B_{12} is believed to increase nitrogen retention is its capacity to increase the utilization of dietary protein and to aid in the conversion of homocystine to methionine in rats [156] Opinions are divided, however on the ability of B_{12} alone to promote growth in instances of methyl deficiency In some microorganisms B_{12} is involved in the synthesis of methionine serine and thymine as well as purines [675]

Administration of B_{12} has been used to counterbalance several conditions that increase protein catabolism Increased nitrogen retention following the administration of B_{12} is important in evaluating changes in lymphoid tissues, for B_{12} increases nitrogen retention in pernicious anemia [540] Either oral or parenteral administration of B_{12} counterbalances the thyrotoxic condition produced in chicks by feeding 0 05 per cent iodinated casein in a corn soybean ration [74] Emerson[28] also found that vitamin B_{12} counteracted the decreased growth rate which follows the administration of desiccated thyroid gland to rats on a diet free of animal protein In addition he reported that there was increased growth of rats which survived thyroparathyroidectomy but that the rate of growth was slower than in intact rats given B_{12} [677] Vitamin B_{12} increased the appetite and growth of male rats which received large doses of thyroprotein or diethylstilbesterol, but

it did not increase the weight of the testes[677] Wang Scheid and Schweigert[10 0] found that decreased spermatogenesis and degenerative changes in the thyroid were counteracted by the administration of B_1 Vitamin B_1 and Aureomycin protected the thymus in rats treated with cortisone and an increase in B_1 also decreased the lo s in spleen weight in rats receiving 1 mg of cortisone daily[677]

The basic importance of B_1 in nucleic acid and protein metabolism is indicated by its ability to increase growth in mammals and bacteria and by its being essential to various processes, including embryonic development as well as hematopoiesis Rege and Sreenivasan (1950) found that adding B_1 to the media increased DNA production by *Lactobacillus case*[1011] Deficiency of B_1 prevents the growth of *Lactobacillus leichmannii, Euglena gracilis* and a *Bacillus coli* mutant,[350] and decreases the cytoplasmic basophilia of hepatic cells in rats[918] The cytological changes in the liver which occur in protein deficiencies are augmented by vitamin deficiencies that decrea e the formation of vitamin coenzyme transformations[55] and thus favor subsequent atrophy[141] and necrosis[384]

HISTONES

Lymphoid tissue because of the lymphocytes it produces is a synthesizing and storing organ for the amino acids in histones Small lymphocytes occur in blood, lymph, tissue fluid, columnar epithelial cells of the intestine[35 7 8] and cerebrospinal fluid They transport DNA and the amino acids as histones Small lymphocytes also contain ribonucleic acid (RNA) amino acids other than those in thymus histone, and other substances in the cytoplasm However, the amount of cytoplasm is a very minor part of this cell The small amount of RNA that the small lymphocyte contains may have a most important physiological function if as is apparently the case, it serves as the source of adenosine triphosphate (ATP) and/or adenylic acid

Infiltrations of small lymphocytes localize and increase the concentration of most amino acids necessary for mitosis of cells in other tissues In 1909 Huiskamp prepared nucleohistone from thymus[699] and Kossel, in 1927 discovered that histones are combined with nucleic acids in cell nuclei[410] in a salt like combination linkage[77] Lymphoid ti sues including nodes thymus and spleen are good sources of histones[60 39] However the thymus is the richest source A 25 per cent yield of histone was obtained from the thymus of man and 27 per cent from the thymus of the ox[977] The high content of histone in lymphocytes has repeatedly been shown by the extraction and precipitation of the chromatin of the nuclei[698 701] Nucleohistone comprised 90 per cent of the chromo omes (chromatin threads of Mirsky and Pollister 1943, residual chromosomes or chromosomes of Mir

sky and Ris, 1947) of lymphocytes, whereas chromosomes from hepatic cells contained only half (45 per cent) as much as lymphocytes

Estimations of the percentage of histones in thymus nucleoprotein vary from 10 per cent, obtained by Pollister and Leuchtenberger,[808] to 30 per cent, obtained by Allfrey and co workers[999] (1952) Chromosomes of different tissues contain varying percentages of histones For example, chromosomes of calf thymus contained 8 5 per cent histone,[999] and Mirsky and Ris (1949) show that chromosomes in calf liver contained 39 per cent histone,[999] and Swift[999] reports that the nuclei of lymphocytes and of hepatic cells have nucleic acid to histone ratios that ranged from 1 2 1 0 to 1 6 1 0

Several proteins in addition to nucleohistones have been found in the nuclei of lymphocytes Mayer and Gulick (1942) found an alkaline soluble protein in nuclei in the thymus of the calf [999] Kirkham (1952) reported the extraction of a globulin fraction from calf thymus which may account for the additional protein described by Pollister and Leuchtenberger (1949) who stated that total protein" in nuclei of the thymus gland of guinea pigs was several times greater than had been assumed from biochemical analysis [999] Mirsky and Pollister[699] describe a non histone protein in chromosomin that contains about 1 per cent tryptophane The chromosomin of Steadman and Steadman[977] has reported to contain arginine tyrosine glutamic acid, asparatic acid and tryptophane From a qualitative standpoint, the controversial status of chromosomin involves only glutamic and asparatic acid because the other amino acids are present in histones, whereas glutamic and aspartic acids are very readily synthesized within the organism [41]

Most somatic nuclei contain histones in salt like combinations with nucleic acid and protein [? 1084] However sperm of certain species contain protamine a simple form [41 977] Nevertheless, histones in small lymphocytes are very important in the metabolism of other cells The small lymphocytes are the mature non dividing and circulating form of this cell They do not utilize the histones which they contain for their own metabolism becau e great numbers of histone laden small lymphocytes disintegrate hourly In addition mitosis in small lymphocytes is very rare and may be partly due to the high content of histones because low histone content has been found in tumor cells and in normal cells in mitosis This relation may indicate that an excess of histones may inhibit cell division [977]

Thymus histone is an important protein because it contains most of the essential amino acids arginine histidine lysine leucine isoleucine threonine, methionine and valine [60 77 11 407 54] The presence of most essential amino acids as components of histones in nuclei of lymphocytes gives added significance to their disintegration in the blood and in tissue fluid of connective tissue The histones in small lymphocytes retain, in an inactive

form, most amino acids necessary for the formation of histones within or covering the chromosomes

Two amino acids which are essential in protein metabolism,[80] phenyl alanine and tryptophane are not usually found in thymus histone A trace of tryptophane in histones was reported by Kossel[410] but Steadman and Steadman[977] state that thymus histone does not contain tryptophane Two non essential amino acids, tyrosine[412] and cysteine, are present in thymus histone[977] The absence of some amino acids in thymus histone may be just as significant in the metabolism of lymphocytes as is the presence of other amino acids Cysteine, glutamic acid and glycine do not occur in thymus histone[41] The absence of these three amino acids may be significant, because they are inhibitors of alkaline phosphatase[993] Alkaline phosphatase, which occurs in all animal cells except hyaline cartilage[870] is very abundant in lymphoid tissue[46 993 104] where it occurs chiefly in the nuclei of the lymphocytes[790] Alkaline phosphatase catalyzes the hydrolysis of orthophosphates from phosphomonoesters and has recently been found to function in transphosphorylation[993]

PROTEIN STORAGE

The "protein storage,' or "amino acid pool is an elusive entity Instead of being a definitely delimited structure such as stored fat, it appears to be a reversible physicochemical process carried on at any site in the body when and where it is needed[11] That there is storage of protein in the various tissues and intercellular and circulating fluids of the body which is available whenever it is needed, is indicated by investigations showing that when 20 to 30 g of plasma protein is lost it is replaced within 6 to 12 hours presumably from fluid withdrawn from the tissues[713] This stored protein in the form of amino acids in combinations amounts to an estimated 2 to 3 kg in a normal person[11]

The idea that protein, as well as carbohydrates and fats but in less quantity is stored in the body is comparatively new Until 1935 physiologists believed that there was very little replacement of protein in living tissue by dietary amino acids and that this was merely 'to compensate for the 'wear and tear' of the body constituents'[3] Folin and Denis (1912) explain the then current view that all absorbed protein that is not used in repair is catabolized and excreted by the kidneys[3] Borsook and Keighley (1935) showed that instead of being inert metabolically dietary protein is highly important in the maintenance of the tissue and plasma level of free amino acids in furnishing much of the material for the synthesis of protein and peptides and in general in supplying the demands of continuous synthesis and degradation of cellular protein[32]

Whipple and co workers (1942) have shown that there is a definite relationship between diet protein and regeneration of plasma proteins and that the "dynamic equilibrium" between the tissue and plasma proteins is to a great extent dependent upon the proper maintenance of diet protein[344] Other works show that hypophyseal growth hormone "facilitates nitrogen retention and protein synthesis", whereas adrenocortical extract increases urinary excretion of nitrogen and potassium[112] By the use of isotopes, it has been determined that the volume of pooled free amino acids in the human body represents 0 5 g nitrogen per kg of body weight[3-] Boothly and co workers believe that hypoactivity of the thyroid gland is conducive to the storage of "deposit protein" and, conversely, that thyroid hyperactivity antagonizes storage of protein and causes breakdown of the excess protein in hypothyroid myxedematous swelling in man[67] The adrenal cortex plays a part in maintaining the plasma albumin, but not the total plasma protein level[600] After adrenalectomy or hypophysectomy, the albumin plasma level falls and the globulin ratio is definitely reduced[600] Thyroidectomy was followed by increased globulin, but it did not affect the albumin level[344]

RELATIONSHIPS OF THYMUS AND GONADS

Involution of the thymus of man during puberty has been recognized for a long time Calzolari (1898) found that castration caused hypertrophy of the thymus and that injection of sex hormones or gonadotropic substances produced involution of the thymus in these same rabbits[159] Gonadectomy or failure of development at puberty delays thymic involution[638] A review by Marine Manley and Baumann[640] indicates that gonadectomy produces or is followed by hypertrophy of the thymus and other lymphoid tissues, but that thymectomy has little if any effect on the development of gonads Removal of gonads is followed by an increase in the size of the thymus[803, 804] and in the weight of lymph nodes[159] However, castration of 50 to 60 day old albino rats was reported to have retarded body growth of males by 25 per cent but it accelerated body growth of the females by 31 per cent[805] Chiodi[159] observed that testosterone propionate produced thymus atrophy in castrated and normal male or female albino rats However more recent studies have indicated that testosterone increases the thymus and other lymphoid tissues Administration of testosterone to normal animals increased nitrogen retention[796] According to Kochadran (1944 and 1950) testosterone reduced urinary nitrogen excretion and increased the deposition of protein in accessory sexual tissue of normal and castrated rats[948] Testosterone propionate[688] and various crystalline androgens[733] maintained spermatogenesis in hypophysectomized rats but caused atrophy of the testis in non

hypophysectomized rats that received testosterone for 7 days[664] Other hormones also cause atrophy of the testis Rats that received thiouracil over 600 days did not have germ development in the testis[168]

The thymus of those hamsters which recovered from a total body x irradiation of 795 r was larger at 33 days after exposure (figs 17 and 18) than the thymus of controls of the same age (fig 16) Since the gonads of this group were atrophied when the animals were sacrificed the increased size of the thymus was attributed to a permenent disruption of nucleic acid metabolism in the testis and ovary, and resulted in the thymus re assuming its prepubertal function in nucleohistone metabolism[5 8 535] The results, thus obtained, parallel those obtained by gonadectomy

EFFECT OF THYMECTOMY

Effects of the destruction or removal of the thymus upon the gonads are not so striking as the effects of castration upon the thymus Destruction of the thymus during the first few days of the rat's life retarded sperma togenesis[930] while x irradiation of the thymus in newborn rats produced reversible aspermia[803] However, Putzu Donnedu[818] found in rabbits that if thymectomy was performed prior to puberty it produced little change in reproductive activity and if it was done during the adult state, this activity was reduced Contrarily Plagge[803] reported that total thymectomy of newborn rats did not alter the growth of the testes, the formation of hormones or spermatogene is but that castration performed prepuberally or at puberty increased the weight of the thymus Removal of the thymus was not found to hasten sexual maturity[639] The more rapid involution of normal and transplanted thymi in breeding rabbits does not necessarily suggest a 'specific nerve influence,'[639] but could be a direct result of increased protein catabolism or decreased nitrogen retention

Other results of thymectomy include changes in various lymphoid organs Parrier found hyperplasia of lymphoid tissue and the formation of large germinal centers in the spleen after thymectomy[645] Magnani also found hypertrophy of the spleen in young and adult rats after thymectomy, and Matti found that primary hyperplasia was followed by atrophy of the spleen after thymectomy[635] Bayer[63] believed that there is mutal compen sation between the spleen and the thymus as indicated by the fact that splenectomy increased thymic efficiency and that thymectomy increased splenic activity The reproductive system would not be expected to be influenced by thymectomy in some species because other normal lymphoid tissues would probably compensate for its function in the storage of nucleo proteins The thymus of rabbits for example repre ents an average of about 12 per cent (11 33 per cent in 5 rabbits) of the total lymphoid tissue,[0] whereas the vermiform appendix represents about 33 per cent

of the total weight of body lymphoid tissue Since appendectomy has comparatively little effect on reducing lymphocytes,[514] the thymus which represents less than half this amount would have very little, if any, demonstrable effect

Some of the controversial effects of thymectomy have been attributed to the presence of parathyroid tissue within the thymus [638] Haberfeld and Schilder (1909) found accessory parathyroids in every rabbit thymus examined [638] Shapiro and Jaffe (1923) demonstrated accessory glands in 12 per cent of a single histological section from the thymus of cats [638] Nicolas and Swingle (1925) demonstrated accessory parathyroids in 35 per cent of their cats, and Farner and Klinger (1920), in nearly every cat examined [638] Some of the early controversial work concerning the effect of thymectomy decreasing growth may be due to the presence or absence of functional accessory parathyroids Marine[638] points out that acce ory parathyroids account for different results following extirpation of parathyroids Among the many varied effects attributed to thymectomy is the defective formation of the shell and albumen of bird eggs [840] Gilmour[343] suggests that defective egg development may be due to the parathyroids being embedded or buried in the thymus Gewers (1930) claimed that thymectomized guinea pigs had defective calcification of the teeth [594]

The high incidence of accessory parathyroids may also account for the reports on the development of rachitic lesions in thymectomized rats[778] and for altered osseous development in various animals following removal of the thymus [963]

Nutritional deficiencies cause atrophy of the testes and involution of the thymus Most deficiencies of B vitamins or amino acids, which cause depletion of the thymus and other lymphoid tissues, also cause atrophy of the testes Nucleic acid formation decreases in the testes as well as in the lymphoid tissue in conditions of deficiency of thiamine or certain other of the B vitamins [117] Thus it is not surprising to find that deficiency of any of the B vitamins inhibits spermatogenesis [97] Jackson[471] reviews the literature on the effects of deficiency of individual B fractions and makes the generalization that 'the testes are especially susceptible to dietary deficiency of vitamin B ' Deficiency of B_{12} for 4 weeks in rats decreased spermatogenesis and produced other degenerative changes of the seminiferous tubules and interstitial connective tissues [1070] Atrophy of the testis has also been produced experimentally by diets deficient in various amino acids including deficiency of phenylalanine [914] threonine[915] and arginine [506]

9

The Spleen

The spleen is an organ for the conversion of extracellular plasma proteins into intracellular protein, chiefly for retention in developing lymphocytes and plasmacytes. Lysis of these cells converts the intracellular proteins into extracellular proteins. This capacity of withdrawing and releasing proteins permits, to a surprising degree, regulation of the amount of plasma proteins transported by the hepatic portal vein to the liver. Specialized vascular arrangements which enable the spleen to store nucleoproteins and to perform these reversible interconversions include the highly specialized sinuses and the systemic hepatic portal vascular relations. Burnett and Fenner's[1,4] view that the spleen is the only organ in which the blood is in direct contact with the tissues of the organ seems to simplify the explanation of this relationship; however, this statement apparently presupposes the presence only of "open" splenic capillaries and may need to be amended.

The spleen is a regulatory or accessory organ, rather than a vital organ, as is indicated by the numerous instances in which splenectomy has not produced any permanent or deleterious effects in men or mammals under normal conditions. However, this statement may be questioned if there are present those conditions which require splenectomized animals to need sorely their splenic blood reserve. For example, it has been shown that when splenectomized and normal guinea pigs were exposed to atmosphere containing known percentages of carbon monoxide, the splenectomized animals succumbed much sooner than the controls. It was found that the normal guinea pigs survived amounts of carbon monoxide which were lethal for the splenectomized animals.[77]

The spleen does not contain characteristic cells, tissues or structures as do most organs. The remarkable splenic sinus has no counterpart, but other structures have smaller and less specialized sinusoids which contain the same littoral cell types that occur in splenic sinuses. The littoral type of reticuloendothelial cells which are present in the spleen also occur in the liver and bone marrow. Lymphocytes, monocytes and plasmacytes are present in most tissues such as the lymph nodes, blood and connective tissues. The distinctive functions of the spleen are due to the facts that blood instead of lymph is the body fluid in contact with these cells in the spleen and that the highly specialized splenic sinuses which are depicted and described

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The concept of the spleen being a protein regulating and storage organ does not preclude this organ from having other functions For example, the red pulp of the spleen serves as a reservoir of blood [77 90] and also of white blood cells and platelets in addition to red blood cells [697] However, stasis of the blood in the spleen favors phagocytosis and plasmacytogenesis In addition, the retention of blood in the spleen is a mechanism for the rapid expulsion of blood For many years the spleen has been recognized as a reserve for erythrocytes [77 454] which can be forced into circulation by contraction of the capsule Anoxemia has been considered a cause for the contraction of the spleen [988] Products of muscular activity have also been shown to cause splenic contraction [703] The affect of exercise may alter the structure of the spleen, for example, dogs trained for endurance running have been reported to have an increase of trabeculae, but only a very slight increase in the muscle layer of the trabecular veins [4 5] Al o, the spleen may *be important as an immediate available source of proteins, proteolytic* enzymes peroxidase and other substances

INTRACELLULAR AND PLASMA PROTEIN SHIFTS

Assimilation of exogenous and endogenous protein was suggested as a possible function of the spleen by Weickel [10 7 1078] The formation of plasmacytes during antibody formation[83] is another function Whipple and associates (1942, 1943) state that there is constant interchange of molecules between blood and tissues in the dog [1084] If such interchange is occurring, the spleen would be a most important organ for this conversion Furthermore the synthesis of nucleohistones by large lymphocytes and the differentiation of some of these stem cells represents intracellular retention of proteins in small lymphocytes a cell which stores and transports nucleoproteins as a "packaged unit Furthermore the distribution and concentrations of lymphocytes and their disintegration is regulated by hormones by the histamine epinephrine balance, and by the balance between the parasympathetic and sympathetic nervous systems The spleen also contains reticuloendothelial cells which are specialized in withdrawing abnormal proteins from the blood [493 1079] as well as abnormal carbohydrates and lipids Granular leukocytes are also involved in carbohydrate shifts in the spleen Wagner (1946) showed that the granular leukocytes were the only blood cells to carry glycogen [1107] Wislocki and Dempsey [1108] narrowed this statement to include only neutrophils The carbohydrase, β glucuronidase and an enzyme responsible for splitting the linkage of the alcohols with glucuronic acid are in blood plasma and are 'present in high concentrations in leukocytes [998] When specific antisera are used mature and immature lymphocytes and polymorphonuclear leukocytes have pecific antigenic substances which connote a difference in chemical composition between

by Knisely,[551, 552] are situated between the arterioles and the hepatic portal venules.

Three well recognized functions of the spleen include (1) storage of blood, (2) destruction of erythrocytes and other blood cells, and (3) the production of lymphocytes in the white pulp.[77] However, these three functions may be considered as facets of the one basic function of its being an organ for regulating proteins in the blood.

Plasma proteins in certain forms of hyperproteinemia are partly withdrawn from the plasma and retained intracellularly within the spleen by the process of lymphocytopoiesis and plasmacytogenesis. Mitosis of large lymphocytes in the germinal centers of the white pulp serves to retain and store nucleoproteins, as lymphocytes are forming. In turn, lysis of these cells releases protein primarily into blood in the hepatic portal vein. The disintegration of erythrocytes and leukocytes in the spleen is also a source of protein which again may be retained intracellularly or pass into the blood of the hepatic portal vein. Quantitatively, the disintegration of erythrocytes in the spleen becomes a significant factor in considering protein metabolism.

There are many reasons for failure to recognize the basic function of the spleen in protein metabolism. In the first place, cells which are present in the spleen occur in several other organs and tissues. Hypertrophy of these cells partly compensates for some of the effects of splenectomy. Some experimental data that were necessary for arriving at the function of lymphocytes and plasmacytes in nucleoprotein metabolism were dependent upon the use of several of the more recently developed techniques such as radioactive isotopes and electrophoretic methods. Furthermore, systemic changes in lymphoid and hematopoietic tissues alter lymphoid tissue in the spleen. Another reason is that certain steps in protein catabolism begin in the spleen, but are completed in hepatic sinusoids; the liver, therefore, is accredited with being the organ in which the function occurs. Furthermore, the spleen changes its function during ontogeny but can reassume hematopoietic functions in certain normal physiological conditions, such as pregnancy in hamsters.[518] Ascertaining the functions of the spleen is further complicated by the wide variations in its significance as a hematopoietic organ in the various classes of vertebrates.

The spleen has been studied by investigators in several fields. Physiologists have stressed its function as a reservoir of red blood cells; hematologists, as a hematopoietic and hematolytic organ; and immunologists, as a source of antibodies. Pathologists often emphasize the 'filter like' function of the reticuloendothelium in the phagocytosis of parasites and removal from the blood of various abnormal or excessive substances, such as cholesterol.

VASCULARITY OF THE SPLEEN

Splenic vascularity and the high degree of specialization of the sinuses are controversial subjects. The specialized morphology of the splenic sinuses is the anatomical basis for the function of the spleen in protein metabolism. The spleen has a very rich blood supply[694] which is characterized by the presence of a specialized venous sinus. A sphincter valve occurs at each end of the sinuses and permits the filling of the sinus with arterial blood and its retention for varying periods of time. This mechanism is germane to the function of the spleen in the interconversion of proteins between extra- and intracellular forms. The arteries enter, and the veins and lymphatics leave the spleen through the hilus. The trunks of the two vessels are usually parallel in the trabeculae. Arteries in the spleen have been considered to be terminal rather than anastomosing vessels[784] and are definitely associated with the white (lymphoid) pulp, whereas veins occur primarily in the red pulp. Infarction due to occlusion frequently occurs, especially in man,[16, 151, 235, 694] because there are very few or no intrasplenic arterial anastomoses.[18, 33, 614, 1039] Arteriovenous anastomoses[813] and arterial capillary venular shunts[5, 1, 793] have been described, but apparently their presence is not universally accepted.

Arteriovenous Connection

A number of investigators who studied the vascularity of the spleen in fixed or living preparations failed to record their finding of this type of anastomosis in this organ,[813] although it has been suggested that arteriovenous anastomoses occur throughout the body. However, Prinzmetal and associates[813] have shown that glass spherules, many times the diameter of the capillaries, injected into one of the splenic arterial branches traversed the spleen in 30 seconds or less, in 5 dogs anesthetized with pentobarbital sodium. The glass spherules had diameters of 160 to 370 μ and were recovered in the splenic vein or liver of all 5 dogs injected. These investigators, thereby, believe that the spleen has direct arteriovenular anastomoses which serve as shunts.

Just how the arteriolar blood passes into the venules in the intrasplenic, or "separatory," circulation[694] has not been determined to the satisfaction of all the investigators concerned with this problem.[16, 293, 551, 5, 4, 681, 694, 746, 793, 1079] The various results form the basis for three principal approaches to the problem of arteriovenous junction in the separatory circulation. (1) In the so-called "closed" approach the arterial capillaries enter the venous sinuses directly, thus forming a continuous tube. (2) In the "open" approach the arterial capillaries are believed to end as open tubes which spill the blood into the intercellular spaces in the red pulp whence it

mature and immature lymphocytes and between mature and immature granulocytes [980]

Many functions of the spleen have special significance because blood goes from the spleen directly to the liver Destruction of erythrocytes in the spleen results in the formation of hematin, hemin and hematoporphyrin, which are normally eliminated in bile, whereas intravenous injections of these substances cause bilirubinemia [10 3] Furthermore, withdrawal of certain substances by reticuloendothelial cells in the spleen deters the retention of these substances by Kupffer cells in the liver The formation of foam cells in the liver blocks the passage of blood through sinusoids in the liver, whereas the formation of foam cells in the spleen occurs in an organ where *there is less interference with the vital functions*

The spleen takes the brunt in the early stages of Gaucher s disease in man [847] In this way it protects the liver and bone marrow for a time, or even possibly effects remission of the disease before the liver becomes irreparably involved The common occurrence of *infarcts*, many showing fibrous replacement in the spleen in chronic Gaucher s disease,[151] may indicate an actual basis for the suggestion that the spleen impedes the progress of Gaucher s disease Apparently, the hepatic involvement begins as a progressive engorgement of the Kupffer cells with kerasin and leads to the disruption of the organization of the hepatic lobules with subsequent anemia and death of hepatic cells [151]

That venous blood from the spleen empties into the hepatic portal vein is most important in considering the functions of the spleen in protein metabolism Catabolic processes which begin in the spleen often are completed in the liver Amino acids freed by proteolysis in the spleen can pass directly to the liver, where they can be decarboxylated or deaminated, or where they may be used by hepatic cells in the synthesis of albumin and fibrinogen Purines and pyrimidines can be oxidized when the splenic blood reaches the liver In addition plasmacytes disintegrate in the splenic and hepatic portal vein[519] so that their content of ribonucleic acid (RNA) and globulin is immediately available for utilization by hepatic cells

Interchange between extracellular and intracellular proteins occurs in all lymphoid tissues The spleen however is interposed between arterial and venous vascular systems whereas lymph nodes are interposed between tissue fluid and blood Extracellular proteins in blood in the spleen may be used by large lymphocytes and then stored and transported intracellularly in the mature differentiated cells which are known as small lymphocytes The process of plasmacytogenesis represents intracellular protein storage that is normally reconverted to utilizable extracellular forms as this cell disintegrates in the hepatic portal vein and sinusoids of the liver [519]

The description of the central artery and its branches which terminate in three types of capillaries was taken chiefly from the work of Knisely[551, 5]

1 The peripheral capillaries of the Malpighian body belong to the conventional type These capillaries form a network around the nodules and supply most of the white pulp and as typical venous capillaries commonly empty into venular tributaries of the trabecular veins

2 The straight or curved capillary shunts are essentially sinusal capillaries which by pass the splenic sinuses by coursing around or between them to empty directly into collecting venules It has been suggested that the function of shunt capillaries is to nourish the tissues during the long storage phases of the sinuses [5 1] The shunt capillaries probably also afford an escape or overflow in the event that excess blood is occasioned by contraction of the valves that control the flow through the venous sinuses Shunt capillaries may have an additional relation to the venous sinuses similar to that in which arteriovenous (a v) connections have to the true capillaries in the mesentery[1138] and elsewhere That is blood may flow continuously through shunt capillaries independently of the amount passing through or detained by sinusal valves

3 The arterial sinusal capillaries are branches of the same arterioles which give rise to the shunt capillaries but they terminate in the venous sinuses

Structure and Function of Sinuses

The simple venous sinus of the spleen is basically dilation of a penicillar capillary and is shaped somewhat like a sweet potato or cucumber with an afferent arterial capillary entering at one end and an efferent venous capillary exiting from the opposite end It empties directly into a venule in living unstimulated spleens[551] Many variations in the modes of interconnection between two or more sinuses occur[551, 93] Bizarre variations in the number and arrangement of afferent and efferent capillaries have also been reported[393]

The wall of the splenic sinus is primarily composed of specialized reticular connective tissue cells[16] and is lined with reticular endothelial cells These endothelial cells are intensely phagocytic[748] but are sometimes said to be less active than the phagocytes in the red pulp[16] The presence of preformed openings (mural stomata)[694] in the wall of sinuses is a disputed point in sinusal structure[16, 11 7] Keyes[574] accepts Mollier's[1911] view that lacunae (stomata) are present in the sinusal walls of lower animals but he points out that there is no morphological proof that mural stomata are present in the splenic sinuses in man Nevertheless blood[16] lymphocytes and erythrocytes[651] pass through sinusal walls but plasma and colloids

eventually wanders, presumably, through mural stomata into the venous sinus (3) In the "mixed' or "compromise' approach, some of the arterial capillaries remain "open,' and others enter the sinuses directly (clo ed), still other arterial capillaries by pass the sinuses and enter the venules di rectly[551, 793]

Arey[16] believes that most of the available evidence appears to favor a closed system Other investigators[5, 1, 694, 746, 793] believe that the closed sys tem of circulation is operative with some modification including leakage Others maintain that the passage of erythrocytes and lymphocytes through sinusal walls is difficult to reconcile with either the open or the closed the ories of circulation[661] However, the great number and specialized archi tecture of the sinuses which apparently give origin to all venules in the red pulp[5, 1] indicate that comparatively few of the arterial capillaries empty directly into venules or veins Historically, it is interesting to note (1) that the basic principles of the so called open system were hypothesized by Bil roth (1862) and several other investigators (2) that Weidenreich[694] (1901), Schweigger Seidel (1863) Thomas (1899 and 1924), Helly (1901 and 1903) and others described or observed the direct entry of arterial capillaries into the venous sinuses in the spleen[574] and (3) that this problem had not been satisfactorily solved as late as 1957[16, 661]

The morphology and much of the physiology of splenic sinuses are de scribed by Knisely[1, 5] who used transillumination of the living, unstimu lated spleen by means of a fused quartz rod His findings on 75 mice, 30 rats and 15 cats were compared with the adverse findings of MacKenzie, Whip ple and Wintersteiner (1941) and were verified experimentally by Peck and Hoerr[793] who accepted Knisely's[5, 1, 55] explanation as probably the correct concept of the circulation as it pertains to the splenic sinuses

MacKenzie, Whipple and Wintersteiner (1941) used 210 mice, 12 rats, *6 rabbits, 8 guinea pigs and 13 cats*[694] *in making a restudy of Knisely s work* These investigators reports categorically denied"[694] and are in "complete disagreement'[793] with Knisely s findings Peck and Hoerr[793] made a re study of both of Knisely s papers and of MacKenzie Whipple and Winter steiner's papers with special emphasis on the technique of *in vivo* trans illumination of the exteriorized spleen by means of a fused quartz rod and of the environmental conditions incident to the experiments Peck and Hoerr found that minute vibrations distorted or completely obliterated the definition of structure under magnifications of or even less than, 600 to 800 times, and that thermal changes of 3 C altered the flow of blood in living unstimulated spleens The account of this restudy makes interesting read ing and, apparently conclusively *establishes the validity of Knisely s find ings*

In general with the possible exception of his failure to observe mural stomata in normal spleens *in vivo*[661] histologists appear to be favorably impressed with the over all applications of Knisely's[31] explanation of the functions of splenic sinuses except that some investigators and writers[16, 393, 483, 551, 65, 661, 10 9] apparently feel that neither the open nor the closed concept is adequate to explain the functions of the splenic sinus Bjorkman[1039] (1947) suggests adding another designation He thinks that the plasma filtering functions of sinuses such as he has demonstrated by the use of injected starch and as Knisely[5 1] observed in living spleens should be classified as 'divided circulation

Changes in Volume

The idea of the spleen as a reservoir for erythrocytes and blood plasma is widely accepted However few investigators have been interested in the spleen as a reservoir for the nucleoprotein bearing lymphocytes or in the less obvious function of blood retention in relation to proteolysis and to the intra and extracellular interconversion states of protein The amount of distention by blood or the sudden rhythmic contraction of the spleen is related chiefly to the activity and amount of smooth muscle which is present in the capsule and trabeculae and which varies in different animals[661] Splenic contraction is very pronounced in cats and dogs[77] but is more feeble in men[10 9]

The voluminous literature on the normal functions of the spleen and its response to excitement administration of hormones drugs chemicals or other agents indicates a wide range in the results that are obtained Some investigators believe that rhythmic contractions of the spleen enhance the movement of blood[7, 483] in the red pulp and of the non cellular components of blood throughout the white pulp[1079] The spleen of the cat is capable of storing one sixth of the blood volume of the animal[77, 746] which includes up to one fourth of the total number of erythrocytes This reserve of cells and plasma may be expelled in response to very strenuous exercise[7] Spontaneous rhythmical contractions of the spleen sufficient to cause waves in tracings of blood pressure have been observed[77] presumably in dogs

The histological nature of the capsule and trabeculae of lymphoid tissue (when present) determines whether these are merely elastic or both elastic and contractile structures Organization of the smooth muscle and elastic fibers in the capsule and their extension into the trabeculae[16, 661] makes the spleen the only lymphoid structure which is capable of an appreciable degree of distention and contractility The capsule of the spleen of many mammals is composed of dense connective tissue formed of elastic and collagenous fibers and a few smooth muscle cells The trabeculae are ex

(but not blood) are believed to pass through the sinusal wall during the filtering stage of sinus activity [551, 793, 1117]

The various functions of the splenic sinus are primarily related to its contracted or dilated condition which is controlled by sphincter valves located in the arterioles afferent and/or efferent capillaries [551] Peck and Hoerr[793] *state that only extreme contractions materially affected splenic* circulation, although irregular splenic contractions were observed in practically all the mice studied However, Knisely[551] observed that during the conduction phase, pulsation of the afferent capillary, sinus and efferent venule frequently occurs Knisely[551] and Peck and Hoerr[793] describe sinuses which vary from only slightly distended with both afferent and efferent valves open and blood flowing through freely, to greatly distended with efferent valves closed and the retained blood cells packed so tightly that individual cells could not be distinguished

The splenic sinus undergoes cyclic activity during which at least three *distinct phases have been described,*[551] in addition to its continuous or conduction phase During the conduction phase the sinus has a diameter about 2 or 3 times that of its afferent (penicillar) capillary Apparently, its function at this time is primarily a tube for the conduction of blood During the filtration filling phase the sinus continually increases in diameter as a result of progressive closure of its efferent sphincter valve, and impounds increasing numbers of blood cells accompanied by loss of plasma and colloids through the walls, until the sinus becomes greatly distended with red and white blood cells so closely packed as to form "cast' of its lumen [551] This storage phase may persist for a few minutes or several hours (up to 10 *hours in a cat)* [1079] *Then the efferent valve may partly open* and cause a delayed or interrupted emptying of the sinus or the valve may open speedily and maximally permitting the sinus to empty suddenly [793]

Retention of erythrocytes in sinusoids of the spleen alters the interface between erythrocytes and plasma and causes the biconcave mammalian erythrocytes to become spherical and thereby more readily hemolyzed [100] It is also believed that the function of sinusoids is to serve as a blood depot [946] *probably serving in the emergency function of the spleen as* a blood reservoir [77]

The splenic sinus appears to be a very important site for the transformation of lymphocytes into plasmacytes It has been shown that plasmacytes in 6 lactating hamsters formed a mean of 24 8 per cent of the white cells in the sinu es but only 9 0 per cent of the white blood cells in the splenic vein and 3 8 per cent of these in the hepatic portal vein The average of these percentages in 4 control *hamsters was 13 0 in sinus, 2 3 in the splenic vein* and 3 0 in the hepatic portal veins [519] These cells were absent in peripheral (jugular) blood in all the animals

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tensions of the capsule that passes into and subdivides the parenchyma of the organ [16 660]

The smooth muscle fibers in the connective tissue capsule and trabeculae of the spleen[16 5 4 660] make possible the slow, rhythmic pulsation like changes in the volume of this organ in normal animals and man [660] Excitement, administration of epinephrine, Pituitrin, pilocarpine or other agents greatly increases these contractions [77] Epinephrine may cause either relaxation or contraction of the spleen [964] When it acts on the dorsal root ganglia, semilunar ganglia and certain terminal structures in the spleen, epinephrine causes active dilation and 'constriction by ganglionic or more peripheral action "[979] The considerable lymphocytosis caused by injecting epinephrine subcutaneously into man or intravenously into rabbits is attributed to the contraction of the spleen [979]

The fact that splenic contractions force large numbers of erythrocytes into the circulation was established in 1927 and 1928 by Barcroft and Poole [51] It is now a generally accepted dictum that strenuous exercise, high altitude sudden fright and certain other conditions cause the spleen to discharge its stored reserve of red cells into the splenic vein [7]

Lymphatic System

The lymphatic system of the spleen is peculiar in that the trabecular and capsular systems are independent of each other[493] although the collecting lymphatics in the trabeculae and capsule drain toward the hilus The lymphatics are poorly developed in the human spleen, where they appear to be limited to the capsule[10 9] and to its larger projections, the trabeculae [661 746 11 7] Jordan[493] states that lymphatics do not occur within the parenchyma of the spleen presumably in man Arey[16] points out that lymphatics may be demonstrated in the white pulp but not in the red However Snook (1946) has shown that deep lymphatics occur in the pulp cords (red pulp) in the monkey and guinea pig,[493] horse, mole and mouse [11 7] Drinker and Yoffey [31] believe that the rhythmic pulsations of the spleen compensate for the paucity or absence, of lymphatics in the splenic pulp and several investigators apparently support this idea [77]

Barcroft and Florey (1928) found that the splenic lymph was colored blue after ligation of the splenic veins and the injection of trypan blue into the splenic arteries In non injected dogs lymph was stained with blood before it entered the thoracic duct when splenic veins were ligated [11 7] Splenic lymph from dogs (anesthetized with methane) flowed at the rate of 0 12 to 0 54 ml per hour and contained an average of 61 per cent as much protein as the blood plasma [11 7] Values for the total protein levels of lymph from the liver were about 81 per cent lungs, 69 heart 65 thoracic duct, 60, cervical duct 48 and skin 28 per cent of the blood plasma level (about 6 20 g per cent of whole blood in dogs under Nembutal anesthesia) [11 7]

SPLEEN AND LYMPH NODES COMPARED

The capsule of lymph nodes is similar to that of the spleen in being a dense connective tissue structure which is composed chiefly of collagenous fibers "with a few fibroblasts" and scattered delicate elastic fibers which form dispersed and more numerous medial networks. The few smooth muscle cells in the capsule are situated chiefly at the sites where the lymphatics enter and leave the node.[660] His (1862), Hellman (1930) and others observed varying amounts and distribution of smooth muscle cells in the capsule and trabeculae.[51] The trabeculae in lymph nodes are continuations of the capsule which form stout, but frequently interrupted, partitions [1, 660] throughout the node and, for the most part, are covered by the reticular framework.[660] The parenchyma of the node occupies the cortical compartments (cortical follicles) and the medullary compartments (medullary cords) which are delimited by the trabeculae.[51] Since the trabeculae are interrupted in places, the cortical follicles and medullary cords may be continuous at these points.[660] The sinuses are essentially loosely meshed subcapsular and paratrabecular (the trabecular sinuses) areas.

This type of architecture practically precludes increased volume of the node as a whole, but it provides for considerable volumetric change in certain individual structures within the node. Numerous investigators, including His (1862), supposed that the smooth muscle cells in the capsule and trabeculae functioned in the "propulsion of lymph" or in expelling lymphocytes from the node.[51] The repeated failure of Drinker and Yoffey [51] to demonstrate contraction of the popliteal lymph node in a dog by perfusion with physiological salt solution, with and without the addition of adrenalin, does not indicate that smooth muscle cells are incapable of constricting afferent or efferent lymphatics at the capsule, or of exerting a mild variable form of pumping action especially upon the subcapsular and other sinuses and/or follicles or medullary cords. Florey (1927) and Martin (1932) observed that the addition of adrenalin to mesenteric lymph nodes of dogs and cats suspended in Ringer's solution usually caused contraction of the node independently of the blood vessels of the node.[51]

Lysis of lymphocytes and plasmacytes in the hepatic portal vein could furnish many substances which are altered by the stress reaction. Contraction of the splenic capsule [51, 54] produces a tidal wave of blood rich in lymphocytes and plasmacytes or their components which is forced into the splenic vein and liver. Kleiner [45] points out that the mechanisms by which the sympathetic stimulation of the adrenal glands augments glycogenolysis is not known.

Deoxyribonuclease (DNAase), ribonuclease (RNAase) and other enzymes degrade the nucleoproteins of these cells and could produce a significant increase in adenylic acid in the liver.[1007] If adrenaline acts on the adrenocorticotropic hormone (ACTH) to increase the release of cortisone,

the extensive cytolysis of lymphocytes [96] would be important sources of RNA and other nucleic acid derivatives for use in the liver and other organs For example, mononucleotides from the nucleoproteins of lymphocytes and plasmacytes may be a source for components of several coenzymes The greater amount of lysis and the systemic distribution of the lymphocytes undergoing dissolution would be significantly increased

Increased blood between the cords in the adrenal cortex[9] could considerably increase the excretion of the adrenocortical lipids The medullary and cortical sinusoids become greatly dilated with blood during acute stress The alarm reaction of the fasiculated zone "may assume an almost hemangiomatous aspect" following "over dosage with folliculoids [9] Increased production of ACTH can cause hyperemia, hypertrophy, hyperplasia and "an increased cytoplasmic granule storage in spite of a simultaneously increased corticoid secretion" within the adrenal glands[9] The foregoing accounts of the results obtained with epinephrine indicate that increasing the amount of epinephrine usually increases splenic contraction This process forces unusual quantities of growth promoting and other essential substances into the splenic vein and thence to the liver

Cells in the Spleen

The spleen contains many kinds of cells, but it does not have a characteristic type of cell In the normal spleen there are monocytes, macrophages (general, histiocytes reticuloendothelial cells, littoral cells, phagocytes, *endotheliocytes histiocytes*), *large lymphocytes small lymphocytes*, plasmocytes fibroblasts and fibrocytes Megakaryocytes and cells of the myelopoietic and erythropoietic series may be present in conditions of extramedullary hematopoiesis Since the terminology, origin and functional significance of many of these cells is subject to controversy, it is no wonder that the function of the spleen has been a mystery

Several minor differences in the kinds of free cells the structure of the capsule the amount of smooth muscle[4 4] and the number of sinusoids, occur in the spleens of various mammalian species For example, megakaryocytes are common in the spleen of many adult mice of various lines[300] but megakaryocytes are absent in adult hamsters except in conditions of extramedullary hematopoiesis[518]

The functions of the spleen depend to a great extent upon the free cells within it Reticular cells and fibers occur in the spleen as well as in the thymus, lymph nodes and bone marrow[64] where they form a framework and network for the retention of free cells However the reticular fibers are considered to have great elasticity and may be able to produce a 'swishing movement of blood[64]

The term 'plenocytes refers to large mononuclear phagocytes[39]

whereas the term "splenic cells" includes monocytes and plasmacytes. Plasmacytes were earlier classed as mononuclear cells, splenic corpuscles, or splenic cells. Plasma cells are abundant in the red pulp of the spleen of man[193, 109] and hamsters.[19] Monocytes are also especially numerous in sinuses of the red pulp.[1, 1]

Reticuloendothelial cells in the spleen have several functions which are especially important because blood from the spleen goes directly to the liver. These cells phagocytize erythrocytes, particles of native or foreign proteins, bacteria, and several substances other than proteins. The intracellular retention of various substances by phagocytes in the spleen favors hydrolysis of these substances and provides a means for their slower release into the blood in quantities which can be excreted or slowly inactivated. 'Foam' cells form in conditions of increased cholesterol (xanthomatosis) or of neutral fats in the blood (essential hyperlipemia).[1, 1] The reticuloendothelial system also concentrates salts of heavy metals, such as lead acetate.[87] Since lead interferes with the activity of many enzymes, phagocytosis of such substances within the spleen prevents serious alterations of protein and other synthetic or metabolic processes in the liver.

Phagocytosis

Phagocytosis in its various forms is extensively utilized as a process in the general physiological economy of the body. The earlier concepts stressed the idea of limited protection of the body by leukocytic and reticuloendothelial phagocytosis of invading organisms, particularly bacteria, escaped erythrocytes and particulate matter.[77, 1103] More recently, the function of digesting and storing substances for use in various aspects of metabolic activity has been added to the earlier concepts of phagocytosis as a method for eliminating obnoxious matter. It has also been shown that phagocytosis is not always a beneficial process, for histiocytic phagocytosis of cholesterol may produce atheromatous lesions in arteries.[509] Both Cameron[134] and McCutcheon[663] agree that all types of cells are probably capable of becoming phagocytes under proper conditions. Rocha e Silva[867] believes that the release of histamine is a common cause of increased phagocytosis which is typical of the primary inflammatory response. McCutcheon[663] intimates that all phagocytes are derived from mesenchyme and Cameron[134] points out that Leo Loeb (1902) demonstrated that homologous epithelial cells were phagocytes in certain conditions of tissue culture followed by implantation into guinea pigs. Liebman[605] states that follicle cells in amphibians function as trephocytes for the viable oocyte but when the oocyte dies these trephocytes become active phagocytes and destroy the degenerating ovum.

Phagocytosis of whole cells converts intracellular protein from the en

and occurs within 2 to 4 days after local injections of nucleic acids[10 3] Ranvier (1900) observed that macrophages (clasmatocytes) and monocytes normally reacted to stimulation by shedding bits of their cytoplasm into the surrounding medium[897] Sabin[887] observed this phenomenon of cytoplasmic pinching off in *monocytes, following stimulation by injection* of a dye protein Also she calls attention to the fact that the etymology of the terms "macrophage" and "clasmatocyte" indicates that this cell is a phagocyte and that it sheds its exoplasm Desquamated mesothelial cells in the peritoneal exudate may become spheroidal, and form bud like protuberances[661] which, presumably, are destined to be shed into the surrounding peritoneal fluid

The significance of the pinched off buds, bits and pieces of the cytoplasm of lymphocytes monocytes various phagocytes, leukocytes and certain other cells may be surmised from the normal contents of these cells A rough idea of the activity and trephone content of phagocytes in general may be obtained from the review by Tullis,[103] who refers to the polymorphonuclear leukocytes functioning as phagocytes as the 'ever present shock troops and compared the interior of a leukocyte to Pandora s Box because of its structure variety of formed elements content of various organic and inorganic chemical substances numerous types of enzymes *and phagocytized substances Also he points out that leukocytes have* a correspondingly very high over all metabolic activity Mitochondria apparently are present in all varieties of leukocytes but may vary in number in the different species[846] The versatility of phagocytes is further enhanced by the finding that the permeability of a leukocyte to water is less than that of an erythrocyte but its rate of exosmosis has been reported to be four times that of endosmosis '[103]

Whether each bit of pinched off cytoplasm contains representative materials from the total content of substances in the cytoplasm of the donor cell or only certain selected constituents is not known, apparently Often we have observed a Feulgen positive particle usually a single clump in buds from lymphocytes Other substances in the various cells that pinch off buds of cytoplasm include various proteins carbohydrates lipids enzymes and salts Some enzymes in cytoplasmic buds have reducing activity others hydrolytic activity and still others nonspecific alkaline or acid phosphatases[1067] Lipase is stored in neutrophils and is essential for the *function* of erythrocytes[916]

Erythrocytolysis

Disintegration of erythrocytes is a quantitatively significant factor in protein metabolism in the spleen Erythrocytes are not primarily trephocytes for they like tissue and tumor cells[97] retain most of their content

of trephones as cell components until these substances are freed by cytolysis. Nevertheless, destruction of erythrocytes is a quantitatively significant factor in amino acid metabolism in the spleen. The importance of the spleen in destroying erythrocytes has been thoroughly reviewed by Kyes,[574] who recognized that the destruction of erythrocytes occurs in the liver in most vertebrates except mammals. Furthermore, the spleen is small in marsupials which are the only mammals in which destruction of erythrocytes occurs chiefly in the reticuloendothelial cells of the liver.

One important method by which erythrocytes are destroyed is phagocytosis. The monocyte is one of several kinds of cells which function as erythrophages. The presence of a few peroxidase granules in monocyte[660] may be important in protein catabolism within the phagocyte. Phagocytosis of erythrocytes has been demonstrated by several experimental methods. Addison observed mononuclear cells phagocytizing washed pigeon erythrocytes which had been intravenously injected into rabbits.[4, 9] Conway[183] also reported erythrophagocytosis by monocytes. However, plasmacytes[1, 9] and neutrophils, as well as monocytes, have been considered erythrophages.[1073] Macrophages (histiocytes) phagocytize fragments or whole erythrocytes[77] and also store hemosiderin in their cytoplasm.[77, 661, 746]

The significance of the spleen in removing erythrocytes from the blood is usually attributed to reticuloendothelial cells.[1079] These cells destroy erythrocytes directly by phagocytosis and indirectly after transforming into monocytes or macrophages. Reticuloendothelial cells in the spleen are believed to phagocytize whole erythrocytes[343] as well as particulate matter and debris of erythrocytes.[493, 1103] The phagocytic activity of reticuloendothelium in the spleen is believed to be greater than in the bone marrow.[1030] However, most of the destruction of erythrocytes was considered by Rous and Robertson to be due to mechanical fragmentation in circulating blood.[1101]

ERYTHROCYTE TURNOVER

The rate of turnover of erythrocytes is important in considering the function of the spleen as a regulatory organ for balance between extra- and intracellular proteins. Results of studies that were designed to determine the life span of erythrocytes are highly variable. The duration of life reported for red blood cells of man includes variations of 30 days.[905] Wintrobe[1103] considers the variations reported for the life span of human red cells and concludes that the most probably correct estimate is about 120 days. Ashby, by injecting differentially compatible cells, found that many of the injected cells survived 25 to 50 days, while some were present 100 days after injection into human subjects.[7] For guinea pigs, the life span

of erythrocytes is considered to be 22 to 28 days with increases to 32 to 38 days following splenectomy,[360] for chickens, 35 days, for pigeons, 34 to 45 days and for ducks, 42 days[8 3] Erythrocytes are believed to have a much longer life than granulocytes which have been reported to have a life span of 72 hours[103] By the method of considering the amount of bile excreted, the percentage of erythrocytes destroyed daily has been estimated to be from 150 to 110 of the total[1103] Turnover of erythrocytes is approximately 3 times the volume of that of the small lymphocytes The significance of lysis of erythrocytes in the spleen is, therefore, a quantitatively significant factor in protein metabolism To some extent, there might be a counter balance in exchange of amino acids from lysis of erythrocytes in the spleen toward mitosis of large lymphocytes and between lysis of small lymphocytes in blood and bone marrow and toward protein synthesis in cells of the erythropoietic series

PROTEINS FROM ERYTHROCYTES

Disintegration of erythrocytes in the spleen is a potential source of amino acids which occur in globin and in several other proteins Hemoglobin does not contribute freely to the protein pool[1088] Furthermore the synthesis of hemoglobin during conditions of hypoproteinemia takes precedence over the formation of plasma proteins[1088] The more rapid depletion of lymphoid tissue indicates that small lymphocytes are a more readily expendable form of protein than that in hematopoietic tissue Lymphopenia and lymphoid depletion develop before anemia in conditions of hypoproteinemia induced by starvation caloric restriction or low protein diets[5 10] or by administration of cortisone[5] Hemoglobin occurs within erythrocytes and is the most abundant protein in blood, usually representing 14 to 16 g per 100 ml of whole blood[41] Hemoglobin represents 28 to 30 per cent of the erythrocyte the stroma, 2 to 5 per cent and water 65 per cent[90] Globin is usually classed as a histone[40 1084] and the globin portion, like the heme group of catabolized hemoglobin is believed to be reused[684]

Interchange between globin and the protein pool involves nine amino acids which are essential for both the synthesis of hemoglobin and of serum proteins in rats[64] Further significance of the interchangeability of protein components is indicated by an increase in serum proteins of hypoproteinemic patients following the administration of 3o5 units of globin[57]

The importance of globin as a protein reserve is also evident in the predisposition of anemic individuals to hypoproteinemia[344] Globin from various species has been considered to contain all of the essential[1103] and several of the non essential amino acids[407] Histones of the disintegrating nucleus of erythroblasts in mammals could also be an intracellular source of all or part of the arginine histidine lysine, tyrosine cysteine, methi

onine, threonine serine, leucine, isoleucine and valine in globins[407] but would probably not be a source of phenylalanine glutamic acid, aspartic acid, glycine, alanine or proline, which are the amino acids believed to be present in globin but absent in thymus histone[407, 1084] One of the main differences in globins and histone is the high content of isoleucine in histone and the low amount in globins[41] However, a great amount of leucine (17 3 per cent) occurs in horse hemoglobin compared with 7 4 per cent leucine in thymus histone[407] There are differences in proteins other than deoxyribonucleic acid (DNA) in nuclei and hemoglobin has even been found in the nuclei of avian erythrocytes but not in other nuclei[109]

Several proteins other than globin have been described in mature circulating erythrocytes of mammals Mature mammalian erythrocytes in contrast with avian erythrocytes have little ability to concentrate amino acids[260] Parpart and Dziemian (1940) show that the membrane of the human red blood cell is composed of an apparently characteristic protein which differs from plasma protein in its amino acid content and also in the amount of cephalin cholesterol and lecithin[343] These red cell "ghosts" or hemoglobin free membranes are often ingested and digested by reticuloendothelial phagocytes chiefly in the spleen and liver[343] In addition, it is known that the cytoplasm of red cells contains calcium chloride chromium dextrose, magnesium, phosphorus potassium sulfuric acid and zinc[345] The stroma of erythrocytes contains a globulin, cholesterol and lecithin in addition to hemoglobin[466] In addition there are a few slight age and species differences in amino acids in erythrocytes The percentage of histidine in bovine hemoglobin is 6 81 ± 0 05 per cent for adults and 6 43 ±, 0 04 per cent for fetuses[10 8] The amount of methionine cysteine and total sulfur also varied in five species of mammals but the amino acids in the protein of the stroma of the erythrocytes did not vary in the different species studied[54]

PROTEOLYTIC ENZYMES

Proteolytic enzymes are most important in the balance of proteins between the extra and intracellular states in the spleen MacFarlane and Biggs[6 6] noted that the balance of inhibitory and proteolytic substances in the blood may be most important in protein metabolism by maintaining the equilibrium between blood and tissue proteins Proteolysis in the spleen is no doubt facilitated by prolonged retention of the blood cells in sinuses within the red pulp In addition the fact that the spleen is a portal organ is extremely important because proteolytic enzymes can act within the splenic sinusoids or pass directly into the hepatic portal vein

In 1905 Cathcart[149] reported protease in the spleen and in 1927 Waldschmidt Leitz and Deutsch[1069] stated that only two proteolytic enzymes

were found in the spleen. One enzyme functioned best at pH 4.0, the other, which had an optimal pH of 8.0, was a peptide splitting enzyme. One enzyme which causes proteolysis has been considered to act at pH 6.7 and to resemble the enzyme found in phagocytes from inflammatory exudates and in macrophages of lymph nodes.[429] However, it differs from leukoprotease of granulocytes which is inactive in an alkaline medium.[429] The proteolytic enzyme described by Hedin and Rowland was called lineo B protease[149] and had maximal activity in acid media.[718] In 1930, Kleinman and Stern[546] reported that leucoprotease from the leukocytes of cows was the same as lineoprotease.

A number of enzymes which hydrolyze nucleoproteins occur in the spleen. Mayor and Greco[6] found that splenic and thymic nucleases from calf spleen and thymus could be differentiated from pancreatic nucleases. Arginase,[877] purine nucleosidases, adenosine, adenosine and guanine deaminases, aminopeptidase, cathepsin and RNAase are also present.[993] Guanase,[885] a nucleosidase that acts at pH 6.5,[544] occurs in the spleen. Nucleosidase, which splits nucleosides into purines or pyrimidines and the carbohydrate,[544] also occurs in tissues other than the spleen. Trypsin is an important enzyme in considering inter- and extracellular interchanges of proteins because it liberates DNA from the nucleoprotein.[1,8] The presence of peroxidase in eosinophils, neutrophils and blood platelets[5] may also be an important mechanism in protein metabolism of the spleen. For example, Soberon[9,8] reported that myeloperoxidase of leukocytes, in the presence of H_2O_2 oxidized uric acid and that H_2O_2 reaction products inhibited the oxidation.

Several proteolytic enzymes are present in lymphocytes.[3,415] Also, mononuclear cells in the pulp phagocytized washed pigeon erythrocytes which were injected into the spleen of rabbits.[4,9]

The spleen has been observed to destroy blood cells selectively,[891] and to possess an enzyme which has a proteolytic effect on leukocytes.[10,6] Osogoe[761] noted the disintegration of lymphocytes in the perifollicular areas of the spleen 5 days after transplanting hematopoietic tissue into the circulatory blood of rabbits. Barnes[4] reviews the literature on various proteolytic enzymes that have been reported to be present in lymphocytes. The amount of protease in lymphocytes is about the same as that in leukocytes.[750] Dipeptidase activity could not be detected in lymphocytes, but it is very active in polymorphonuclear leukocytes.[3,5] Waksman and Davison[1063] compared the optimal pH for the activity of a proteolytic enzyme which they obtained from large mononuclear cells and named lymphoprotease with a protease from leukocytes. Lymphoprotease is considered to be active at an acid pH in contrast to leukoprotease which has an optimal pH in alkaline media and is identified as the proteolytic enzyme

from large mononuclear cells Cathepsin, adenosinase nucleinase and purine nucleosidase have been found in lymphocytes[8] It is interesting to note that the optimal pH for chemotaxis of the so called "round cells" is the acid range of 5 9 in contrast to an alkaline pH for leukocytes[103] Grob[373] states that the polymorphonuclear leukocytes of the cat show proteinase and peptidase activity at neutral pH

DNAase and RNAase are present in the spleen and other portal organs Kurnick and Carrera[572] found that the blood in the portal vein of normal rats showed more DNAase activity than that in the hepatic vein, and that bile also contained about the same amount of DNAase as the hepatic vein in normal rats The presence of DNAase in the spleen has been considered in studies on the protective effect of DNA following irradiation[178] Cole and associates[179] consider DNAase substrate as the protective factor in bone marrow of rats and state that it is similar to the protective factor in mouse spleen Douglass and colleagues[41] found increased activity of DNAase in the spleen at 6 12 and 24 hours after total body x irradiation of 800 r but a return to normal by 48 hours in rats The presence of DNAase in other portal organs is important because blood from these organs also goes directly to the liver Possible differences in DNAase from various tissues have been considered by Webb[10 a] who mentioned that pancreatic DNAase may act on cells which have not autolyzed whereas DNAase from the thymus and from leukemic patients requires autolysis of the cells to free the substrate The activity of proteolytic enzymes in the spleen and hepatic portal venous system could explain Kaznelson's tenet that splenectomy is valuable in those cases of purpura hemorrhagica which are alleviated by splenectomy because platelets are being destroyed more rapidly than normal[1103] The effect of splenic extracts in limiting the growth of blastoma was attributed by Bruda[119] to the reticuloendothelium Gaetani[332] states that the histological examination of the spleen and liver of rabbits injected with mouse tumor showed oncolytic powers that were augmented by previous injections of mouse carcinoma This relation may be attributed to lymphoid tissue having a function in resistance to tumor growth Proteolytic enzymes in the spleen however are also a cause for effects of the spleen on cancerous growth Proteolytic enzymes in lymphocytes and other cells in the spleen act on foreign or other proteins and therefore, can camouflage the function of lymphocytes and plasmacytes in the growth of other cells

PROTEIN STORAGE

The spleen is a portal organ which stores protein It forms an important part of the protein pool by being a reservoir for blood and by retaining nucleoproteins intracellularly, primarily in lymphocytes and

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The cells in the spleen which synthesize and store nucleoproteins and gamma globulin probably also free amino acids which occur intercellularly but are not rapidly depleted by starvation[160] Small lymphocytes plasma cytes, monocytes, histiocytes, Kupffer and other reticuloendothelial cells retain free amino acids as well as the nucleic acids, histone, globulins and other proteins

Small lymphocytes and plasmacytes in the spleen synthesize and store *nucleoproteins, but their function in storing free amino acids has not been* established Cytoplasmic levels of many amino acids fluctuate with extra cellular levels[160] The ability of various cells to store free amino acids has not been determined but a nucleus is apparently essential because the mammalian erythrocyte has little ability to concentrate amino acids compared with the great ability of avian erythrocytes which are nucleated[160] Inhibition of intracellular storage of free amino acids may be a contributing factor in ' anemia aciduria which is defined by Harris[405] as the presence of one or more increased amino acid in the urine On the other hand, it has been suggested that increased amounts of intracellular proteins are related to cloudy swelling Virchow suggested that "albumin granules in cells were due to the inability of the cytoplasm to assimilate protein The cause of cloudy swelling has been attributed to altering intra cellular breakdown of protein by various factors[713]

The synthesis of histone by large lymphocytes in the Malpighian bodies of the spleen is especially important because some of these lymphocytes differentiate to the type identified as the small lymphocyte These cells represent intracellular storage and transportation of most essential amino acids in addition to DNA and a small amount of RNA The similarities of histones prepared from various organs of an animal give greater significance to the functions of small lymphocytes because histone in the nucleus of small lymphocytes is a 'packaged unit which carries a group of amino acids for use by cells in other tissues Crampton and co workers[217] found that histone from the calf thymus is the same as histone from the calf liver or kidney in amino acid composition and in chromatographic behavior

The ability of large lymphocytes in the spleen to "withdraw individual amino acids from blood and to synthesize histone intracellularly is a mechanism which is advantageous for transportation and localization of these amino acids as a 'packaged unit" Christensen[160] has emphasized that the deficiency of one amino acid can be tolerated only to the degree that other amino acids can be retained without destruction " The synthesis and storage of amino acids as histone in the nuclei of lymphocytes in the spleen retains most of the essential amino acids in an ideal location for use by hepatic cells in the synthesis of albumin and fibrinogen Further

plasmacytes The direct availability of this "protein pool" for use by the liver has been recognized in various studies of protein metabolism By using isotopes, Sprinson and Rittenberg calculated that the total protein pool respresents 0 5 g/kg body weight in man and that it is regulated by hormones[3] Plasma proteins are one source of the "protein pool," as indicated by Whipple and colleagues who maintained nitrogen equilibrium by intravenous injections of plasma proteins as the only source of nitrogen[117] The significance of lymphoid tissue as an intermediary storage of protein in the spleen has not been considered directly However, it has been recognized indirectly by varied responses, such as (1) the depletion of lymphoid tissue in starvation (fig 21), (2) other forms of protein deficiency (fig 22), (3) in hyperplasia resulting from conditions of hyperglobinemia or (4) other forms of hyperproteinemia

The main storage of protein in the spleen is by mitosis of large lymphocytes to form small lymphocytes in the white pulp (fig 19) The small lymphocyte is a mature cell which does not divide thereby, it can retain and transport nucleoproteins By transformation, plasmacytes develop from some lymphocytes in the peripheral areas of the white pulp and throughout the red pulp The plasmacyte is a synthesizing and storage cell for RNA and gamma globulins, as well as for nucleoproteins

The plasma proteins are considered a source of protein for use by hepatic and other cells However, there are several other sources of proteins in the spleen The destruction of cells by proteolytic enzymes in the spleen increases the amount of extracellular protein which may be utilized by large lymphocytes in mitosis, or by lymphocytes transforming into plasmacytes The breakdown of erythrocytes in the spleen represents the conversion of proteins from these cells into their constituent amino acids, some of which may be reutilized for the synthesis of other proteins in the spleen, while others are variously utilized by the liver In addition reticuloendothelial cells in the spleen are phagocytic and may function in resynthesis and storage of protein, and have a regulatory effect on plasma proteins in the splenic sinusoids and hepatic portal vein For example, cells of the reticuloendothelium as well as lymphocytes and plasmacytes are believed to produce abnormal proteins as indicated in conditions of macroglobinemia which occurs in many diseases[487] Thus histiocytes and monocytes may have important functions in protein storage, as indicated by variations in their content of cytoplasmic RNA This point would be most difficult to determine because these cells are not aggregated in a tissue or organ and it is more difficult to determine their functions However Jansco and Jansco Gabor[480, 481] reported the storage of protein in histiocytes in the skin of mice and rats following subcutaneous injection or topical application of histamine

tributary of the hepatic portal vein where these cells speedily disintegrate in normal hamsters.[519] Many plasmacytes are normally formed from lymphocytes in the spleen, because the venous sinuses of the spleen form sites of pooling or stasis of blood. Stasis of blood, lymph and tissue fluid is favorable for the transformation of small lymphocytes into plasmacytes in hamsters.[519]

One function usually ascribed to plasmacytes in the spleen is the formation of antibodies. Plasmacytes (plasma cells) are usually considered the primary source of antibody because their numbers increase in lymph nodes during antibody formation, and because antibodies can be extracted *from some tissues in which plasmacytes increase after injections of bacteria.*[406] Precipitins and agglutinins[71] are also considered products of plasmacytes. Plasmacytes were also considered an important source of antibody and serum globulin by Fagraeus[1 4] (1948). Serum antibody[91] formation is paralleled by an increase in plasmacytes.

Plasmacytogenesis is especially significant in the red pulp of the spleen because disintegration of plasmacytes in the splenic sinuses and hepatic portal vein provides RNA and DNA and/or their components and, also *the amino acids in histones and globulins for use by hepatic cells.* The disintegration of plasmacytes begins in the venous sinuses in the spleen and is nearly completed before the splenic blood reaches the liver. Plasmacytes in the spleen, however, do not represent the only source of nucleoproteins for use by hepatic cell metabolism because the core of villi, where plasmacytes are abundant in many species, is also a potential source of plasmacytes.[534]

Lymphocytes and plasmacytes are abundant in the mucosa of the stomach, small and large intestines and also in "milk spots" and lymph nodes in the mesentery as well as in the spleen. Blood from other portal organs is indirectly important in protein metabolism of the liver. For instance, the pancreas, as well as the spleen, produces DNAase and RNAase which are necessary for the hydrolysis of nucleic acids into nucleotides. The high number of plasmacytes in the venous sinuses in the spleen of Syrian hamsters (controls 13.0 per cent, lactating mother 24.8 per cent of the total leukocytes) and the progressive dissolution of these cells in the portal system as the blood flows from the spleen toward the liver indicate a direct relationship to the control of plasma protein by the liver. The disappearance of plasmacytes in the peripheral blood[519] and their distribution in the connective tissues of the salivary glands and other organs, indicate that these cells synthesize and store RNA and that their disintegration provides the localization of RNA and globulin for the metabolism of other cells.[53]

In order to determine the fate of splenic plasmacytes in hamsters we[519]

more, the synthesis of cytoplasmic protein during plasmacytogenesis is a source of gamma globulin The abundance of RNA in the cytoplasm of plasmacytes, and the high nucleocytoplasmic ratio of small lymphocytes, are significant in relation to Gale's[333] hypothesis that specific combinations of nucleotides within the nucleic acid furnish points for combination of acids

Cortisone depletes lymphoid tissues and destroys systemic lymphocytes and plasmacytes (figs 22, 25 to 28) [06] apparently, by stimulating protein catabolism[419] This effect of cortisone may be partly overcome by the administration of "large doses of vitamin B_{12} "[678] However, Albright, in 1943, advanced the hypothesis that cortisone inhibits the synthesis of proteins rather than accelerates protein catabolism "[464] A striking effect of cortisone on the liver is that it prevents the pronounced hepatic infiltration of plasmacytes and lymphocytes which accompanies the growth of malignant tumors in hamsters [06 5 0 6 4]

A reversible decrease in basophilia occurred in hepatic cells in the liver of rabbits,[1099] and a decrease of DNA in nuclei as determined by nuclear counts of liver homogenate was reported in liver cells of mice that received cortisone[48] However a decrease in RNA was not reported The failure to detect a decrease in RNA of the hepatic cells in these mice may be due to the short time (5 days) that the cortisone was administered[48] Lowe and Williams[618] reported that 25 mg/day, for 5 days, reduced the RNA in the mitochondria and microsomal pellets in the liver cells of rats

Light chronic injections of cortisone slowly depleted the lymphoid tissue in the Malpighian corpuscles of the spleen (fig 22) and in turn the number of plasmacytes in the red pulp in otherwise normal hamsters Coincidentally with this process there was fatty infiltration of the liver and depletion of the lymphocytes and plasmacytes in the mucosa of the intestine [06 5 0] Seven to 21 injections of cortisone acetate (Merck 1 5 mg/100 g body weight) over a period of 10 to 38 days depleted the lymphoid tissue in the spleen of 10 Syrian hamsters and produced a decrease in the cytoplasmic basophilia which was primarily related to the decrease in RNA The decreased basophilia was followed by fatty infiltration that extended throughout the hepatic lobule[5 0] However this work does not determine whether the fatty infiltration and decrease in RNA in hepatic cells was caused by a decrease in plasmacytes or was the result of a concomitant change due to increased protein catabolism—or, yet, to the summation of the combined effects of these two possibilities

PLASMACYTES IN THE SPLEEN

Normal systemic blood contains few to no plasmacytes in man[1103] and hamsters,[519] but plasmacytes are numerous in the blood in the splenic

resembling those in the description given by Bohm and co workers[91] of the first plasma cells observed by Waldeyer Occasionally Russell's bodies[6-7] were observed in plasmacytes in lymph nodes and other organs but cells that contained these bodies did not occur in the blood vessels of the spleen, or in the portal or jugular vein in any individual of the five groups of hamsters[519]

Plasmacytes occur in the liver of tumor bearing hamsters These plasma cytes may develop from lymphocytes in the periportal periductal connective tissue as a response to increased globulins Janold and Wilter[48] reported correlations between plasmacytosis and hyperglobinemia in the sternal bone marrow Infiltrations of lymphocytes and plasmacytes in the liver of mice having carcinoma and also in hamsters bearing implants of several lines of sarcoma and carcinoma may account for the high activity in the DNA fractions of the liver and spleen of tumor bearing mice injected with C^{14} labelled formate or glycine compared with the activity in the control group[788] In addition, there is the possibility that histamine increases and causes contraction of the muscle in the hepatic veins of hamsters, as it does in dogs and rats[1048]

Depletion of Lymphoid Tissue

Most physiological and pathological conditions have the same effects on lymphoid tissue (white pulp) in the spleen as on lymphoid tissues in lymph nodes, the thymus and other lymphoid structures Changes in the amount of lymphoid tissue usually parallel changes in plasma proteins Normal or pathological conditions which increase protein catabolism or decrease available proteins deplete lymphoid tissues Aging starvation protein low diets cortisone and ACTH decrease lymphoid tissue in the spleen and in other tissues and organs figs 21 22 26 and 28) Experimentally induced hyperplasia which was produced by the injection of foreign proteins Addison s disease or conditions which cause hyper globinemia increase lymphoid tissues in the spleen and also in other organs These parallel effects support the tenet that lymphocytes in the spleen represent an intracellular protein storage which is interposed in the blood vascular system

Lymphoid tissue in the spleen is depleted in the same conditions which cause the depletion of lymphoid tissue in other organs Lympohid tissue in the spleen involutes with aging after puberty in mice[890] and cats[739] The maximal percentage of lymphoid tissue in the spleen of 300 persons who died from violent causes occurred during the 1st decade, then decreased and sharply declined during the 2nd decade to a level which persisted through the 7th decade[4 9] The effect of age on the spleen is similar to its effect on other lymphoid tissues[4 9] Keuenhof[538] reported

appropriately ligated, excised and fixed the thoracic postcava and jugular veins, and fixed the spleen and gastroenteron including the intact hepatic portal vein and hepatic venous systems of 4 control, 16 pregnant, 6 post partum, 8 syngenesioplastically implanted with homogenized, sterile tissues of fetuses, and 16 of the same strain of animals bearing an advanced transplant of a pleomorphic sarcoma (HS 6) After about 2 minutes in the fixing solution, the viscera were removed with ligatures intact, and pieces of the left and right anterior (cystic) lobes of the liver were excised to insure better penetration, and fixation was resumed The tissues were fixed in sublimate alcohol sectioned in tissuemat and stained with toluidine blue This combination of fixing and staining was found to be superior for demonstrating cytoplasmic basophilia

Plasmacytes were numerous and formed and appreciable percentage of the total white count in the splenic sinuses of hamsters in all experimental groups The percentage of these cells progressively decreased as the blood flowed toward the liver and nearly reached zero in systemic blood from the jugular vein The splenic sinuses of the 6 lactating hamsters had the highest mean percentage of plasmacytes (24 8) of any group of hamsters examined The mean percentage of the other four groups was 14 8 in the splenic sinuses of the 16 pregnant individuals, 13 0 in the control group, 16 1 in the 16 hamsters bearing transplanted sarcoma for 18 to 71 days, and 15 1 in the 8 males that received injections of triturated freshly killed, fetal tissue and were killed 3 to 15 days later The presence of extra medullary erythropoiesis and myelopoiesis in the spleen of pregnant individuals did not alter the percentage of plasmacytes in the white cell counts

Several cytoplasmic and nuclear variations were found in plasmacytes within the circulating blood of normal hamsters however, pycnosis was the only stage in the disintegrative process that could be definitely identified The absence of cytoplasmic basophilia in the plasmacytes was due to a decrease in RNA as indicated by the RNAase test of Stowell and Zorzoli[987] Cytoplasmic vacuoles were more numerous in plasmacytes in the blood than in cells within lymphoid organs Cytoplasmic vacuoles and atypical vacuolization have been considered as evidence of protein secretion but the question of whether all plasmacytes or only those in the medulla of lymph nodes produce gamma globulin has been posed[597] Bing[80] believes that the formation of globulin takes place in plasmacytes Ehrlich and associates[274] stated that plasma cells may produce beta globulin and related protein

A few plasmacytes in the blood had numerous small vacuoles similar to those described in Turck s irritation cells[10, 1] and in proplasmacytes[5, 9] Other plasmacytes contained one or several large cytoplasmic vacuoles

sodium caseinate This effect of foreign protein in increasing mitosis could be interpreted as an indirect effect of the foreign proteins Reticuloendothelial cells, monocytes and histiocytes ingest foreign proteins which are retained by the stasis of blood in the splenic sinuses[551] The retention of the foreign protein concentrates the irritant or products of proteolysis such as nucleoproteins and possibly, could alter the permeability of the lymphatics, blood capillaries or blood sinusoids In turn an increased permeability increases the proteins in lymphoid tissues and thereby increases the mitosis of large lymphocytes in 'germinal centers' This indirect effect could also be a part of the mechanism conducive to antibody formation

Wiseman (1931) found that parenteral administration of protein produces lymphoid hyperplasia[134] Rats retained 17 to 41 per cent of the nitrogen intake from egg albumen[646] and were more resistant to foreign protein Injected foreign proteins were used as efficiently by those rats that received it intraperitoneally as by those that received it orally[646] Cramer and associates[14] considered that parenterally injected proteins were ingested and carried in the blood by lymphocytes

Several forms of purpura also indicate that there is a direct relationship between plasma proteins and lymphoid tissue in the spleen Germinal centers in the spleen are large and active in purpura hemorrhagica[100] Descriptions of changes in lymphoid tissue and in the plasma proteins are rarely mentioned in descriptions of the purpuras One type of purpura however is specifically designated by Tager and Klinghoffer (1943) as Minots purpura hemorrhagica with lymphocytosis[1103] Several changes in plasma proteins have been reported in cases of purpura A great increase of globulin which had mobility at pH 8 indicates that beta globulin was present in the serum of a patient with purpura[6 3] Von Horst[1064] found hyperglobinemia in a patient with idiopathic purpura in whom there was also damage of liver cells

An increase in lymphoid tissue and in the size of the spleen occurs in many diseases of the liver This hyperplasia of lymphoid tissue has not been identified with protein dysfunction in the synthesis of albumin and fibrinogen There are of course mechanical causes of splenomegaly which result from pathological changes in blood vessels in the liver The spleen enlarges in some cases of portal cirrhosis and chronic valvular disease[100] part of this increase is attributed to hyperplasia of the reticulum in the spleen

The term splenic tumor is frequently used to indicate an enlarged spleen and does not indicate the cause of splenomegaly However splenic tumor has been attributed to a variety of controversial causal agents and the contradictory explanations of the hyperplasia range from supply

hypoplasia of the spleen in a 72 year old woman, but attributed this effect to a congenital factor and not to age However, atrophy of the spleen is very common in old age [100 981 11 7] Lymphoid tissue in the spleen of man is best developed between the ages of 1 and 20 years, and is arranged as a continuous lymphoid sheath around the arteries [4 1] Depletion of lymphoid tissue in the spleen has been attributed to hyalination of the inner net of the capillary zone, which forms four branches of the follicular artery, in contrast with the outer capillary zone which is derived from arterial loops from the red pulp [4 7]

The spleen is not rapidly depleted during puberty as is the thymus [180] but simultaneous regression of both the spleen and the thymus may occur in several conditions Decreased nucleoprotein metabolism of spermatogenesis is associated with an increase in the lymphoid tissue in the spleen as well as in the thymus, and was reported as the cause of hyperplasia of the white pulp in the spleen of gonadectomized male and female rats [590] This hyperplasia however might be a result of the decreased thyroid activity which also occurred in these rats [90] because, often, hypothyroidism produces hyperplasia of lymphoid tissue in the spleen and in other lymphoid tissues

Wasting diseases, including hemolytic jaundice,[1018] prolonged hunger,[471] or the continuation of most lymphocytopenic agents or conditions deplete the Malpighian bodies and cause atrophy of the spleen Jackson[471] prepared an excellent review of the literature through 1924 on the effects of malnutrition fasting and inanition as the causes of atrophy of the spleen and other lymphoid organs Atrophy of the spleen following total inanition, was reported in dogs and rabbits by de Martigny as early as 1827 [471]

A decrease in the size of the spleen followed repeated doses of 8 azaguanine [309] Hypoplasia of the spleen, as well as the thymus, was caused by dermal operation or the injection of tar whereas the u e of tar for over 30 days retarded growth and caused hypoplasia of lymphoid tissue [31] Lira[611] described a decrease of white cells in the spleen and leucopenia following x rays He also noted that recovery from leucopenia produced by benzene is slower in splenectomized dogs than in norm il dogs

HYPERPLASIA OF LYMPHOID TISSUE

An increase of mitoses of large lymphocytes has been reported in the spleen after injections of various foreign proteins into experimental animals This increase in mitosis has been considered to be a source of, or related to increased antibody formation [51 11 7] Drinker and Yoffey [1] state that there is no question of the effects of foreign protein in stimulating lymphoid tissues and that Oliver and Katzman (1938) found that near leukemic like conditions were produced in mice by injections of

after removal of an implanted HS 6 tumor that had a duration of 23 days[5 0]

Extramedullary hematopoiesis caused splenomegaly, with absence of the lymphoid tissue, in the spleen of a hamster which had carcinoma of the skin induced by 21 topical applications of 9,10 dimethyl 1,2 benzanthracene over a period of 97 days[5 0] Similar development of extra medullary hematopoiesis, accompanied by an absence of lymphoid tissue in the spleen, occurred in a hamster having a sarcoma induced by 9,10 dimethyl 1,2 benzanthracene[5 0]

Cortisone decreases protein anabolism and increases protein catabolism Depletion of lymphoid tissue in the spleen (fig 22) and other lymphoid organs occurs following the administration of cortisone In addition, the administration of cortisone to hamsters prior to and following implantation of a mixed cell sarcoma markedly decreased the white pulp of the spleen and prevented the development of extramedullary hematopoiesis [06] The effect of cortisone in depleting proteins apparently inhibited the development of extramedullary hematopoiesis in tumor bearing hamsters One explanation of the effect of cortisone on lymphoid tissue in the spleen might be that the decrease is due to lymphopenia in the blood, and the consequent decrease of lymphocytes in the spleen However the tenet that lymphoid tissue stores nucleoproteins and that cortisone significantly increases protein catabolism would indicate that the decrease of white pulp in the spleen and systemic lymphopenia are due to the depletion of protein The absence of extramedullary hematopoiesis in the spleen of cortisone treated hamsters that were bearing implanted sarcoma and the development of small hematopoietic foci and white pulp in the spleen of these tumor implant animals when cortisone was withheld for a week,[5 0] indicates that the changes are related to increased protein in the blood after withdrawing cortisone

SPLENECTOMY

Several effects which usually follow splenectomy could be the results of decreased proteolysis Splenectomy has been used by many investigators in their efforts to determine the function of the spleen However, Krumbhaar[567] points out that splenectomy does not indicate the function of the spleen because the elimination of a considerable amount of the total reticuloendothelial system is soon masked by compensatory activity of the reticuloendothelial structures in the bone marrow and lymph nodes

Several changes develop in the liver following splenectomy An increase in reticuloendothelium of the liver often follows splenectomy [1009] Kupffer cells hypertrophied and became actively phagocytic in the liver of guinea

ing necessary elements for the blood to excessive erythrolysis MacCallum[67] apparently considered the use of the term splenic tumor, especially "acute splenic tumor," as being a non committal phrase which fails to indicate either the cause or the processes involved in the formation of splenic tumor Richter[847] expresses similar views concerning the use of the term "splenomegaly" Jawein (1900) associated splenic tumor with those infections and intoxications in which much blood is destroyed (as in typhoid fever), and concluded that the enlarged spleen is related to extensive phagocytosis[6 5] Bernhardt (1913) attributed the splenic tumor in scarlet fever and typhoid fever to the results of phagocytosis of the enormous numbers of accumulated blood platelets[6 5] However, MacCallum[6 5] discredits phagocytosis as a significant causal factor by pointing out that phagocytosis within the red pulp is inconspicuous in enlarged spleens of septicemic patients, but that the greatly swollen spleens of typhoid patients do show extensive phagocytosis of cell debris and erythrocytes

EXTRAMEDULLARY HEMATOPOIESIS

An increase in protein synthesis by hematopoietic cells and rapidly growing tumor cells, diverts the extracellular proteins from storage in lymphoid tissue Lymphoid tissue in the spleen of hamsters decreases when there is advanced extramedullary hematopoiesis in the spleen during pregnancy (fig 20)[518] Originally, this depletion of lymphoid tissue was attributed to replacement by hematopoietic cells, but it could be due to the use of proteins by erythropoietic and/or myelopoietic cells The storage of nucleoprotein by lymphocytes is below normal, although the spleen is twice its normal size as a result of extramedullary hematopoiesis in hamsters killed during the 12th day of pregnancy[518]

Lymphoid tissue decreases in the spleen of hamsters that have implants of a pleomorphic cell sarcoma (HS 6) as extramedullary hematopoiesis increases in the spleen (fig 23) For example, hematopoietic foci were present in the red pulp and a near normal amount of lymphoid tissue remained in the spleen of a hamster having an implant of a mixed cell sarcoma for 28 days[08] However a very small amount of lymphoid tissue remained in the spleen of a 190 day old hamster which bore a transplanted sarcoma for a period of 52 days and no white pulp was found in sections of a hamster which had implants of the same line of sarcoma for 77 days[518] Total extirpation of this sarcoma caused regression and the complete absence of hematopoietic tissue in the spleen —and regeneration of lymphoid tissue in the white pulp (fig 24) A normal amount of lymphoid tissue was present in the spleen of a hamster killed 142 days

on leukocytes For example, leukocytosis with lymphocytosis follows the administration of adrenalin to splenectomized guinea pigs[307] However leukocytosis produced by injections of sodium nucleinate was higher after splenectomy than in the controls[1136] Splenectomy had an ameliorating effect on neutropenia in 4 cases, but had no effect in 2 others[838] Splenectomy modified the neutropenia in a 62 year old man who had cyclic agranulocytosis[3 6] Splenectomy had a beneficial effect on 2 cases of Felty's syndrome, one of which had hydroplastic sternal marrow whereas the other had hyperplastic sternal marrow[95]

Lymphocytosis usually follows splenectomy and could be due to the decreased rate of lysis of lymphocytes which normally occurs in the spleen The increase in lymphocytes persists for 4 to 12 months according to Whitney (1928)[1] Lymphocytosis following splenectomy, may be due to a single factor or a combination of several enzymes which destroy lymphocytes in the liver The spleen may condition lymphocytes so that they are more susceptible to the action of proteolytic enzymes In addition, the spleen may furnish an enzyme or other mechanism for the activation of trypsin or the inactivation of the trypsin inhibitor Krumbhaar[67] observed cellular hyperplasia in the bone marrow after splenectomy, and concluded that this condition represents a compensatory response to anemia and the loss of an important hemolytic organ He suggests that splenectomy may possibly cause lymphocytosis by increasing the activity of bone marrow

A possible cause of lymphocytosis and several changes reported after splenectomy may be a result of the decreased retention of eosinophils in the spleen The spleen functions as a reservoir of eosinophils as well as erythrocytes[359] Furthermore the eosinophils are readily expelled into the blood by epinephrine[3 9] If peroxidase in granules from eosinophils are important in several steps of protein catabolism[illegible] an absence of storage of these cells in the spleen may be a cause of some of the reported changes Several aspects of protein metabolism have been reported to be altered by splenectomy An increase of amino acids has been reported in plasma along with a decrease of amino acids in corpuscles[1034] Formation of creatinine from creatine in the liver is considered to be retarded but formation of creatine was not altered[641] Increases or other changes in globulin following splenectomy, may be a cause of the leukocytosis as indicated in Menkin's statement that the factor which promotes leukocytosis is either a globulin or globulin fraction of exudates[68]

Effect on Erythrocytes

Several changes in erythrocytes and their development which follow splenectomy may be due to decreased proteolysis in the spleen The reticulocytosis which often follows splenectomy has been attributed to increased red cell longevity which increases in normal guinea pigs from a

pigs splenectomized 10 weeks previously [465] Several other changes which have been directly attributed to splenectomy may be due to indirect changes in the liver For example, splenectomy has been considered to diminish tolerance for carbohydrates [1054] but this reaction may be due to other compensatory effects on the liver

Splenectomy in rabbits has no marked effect on the remaining lymphoid tissue The lymphoid tissue in the spleen comprises a very small percentage of the total lymphoid tissue in the body in rabbits and its absence can easily be compensated for by the increased activity of the remaining lymphoid organs as has been reported in the rat [446] The effect of splenectomy in the rabbit upon the amount of the remaining lymphoid tissue agrees with that in the guinea pig,[1 7] dog[903] and man [514 929]

Splenectomy, alone or combined with appendectomy, did not produce marked variations in the growth curve of young rabbits nor did this deviation differ from appendectomy alone Loss of weight in 1 rabbit, following ablation of the spleen and appendix was less than 200 g and lasted less than 1 week These results are contrary to those reported by Montemartini[710] who states that a decrease *in weight persists for a period* of several months after splenectomy and with the results of Soula[963] who concluded that splenectomy affected growth in rabbits through an insufficient supply of lipids [514] Bardeleben (1841) found that one of the effects following splenectomy is "an increase in activity of bone marrow and lymphatic glands" [45]

Decreased antibody formation[1110] is a result of splenectomy Rowley[883] found that splenectomized animals formed a low circulating antibody titer if a small amount is injected intravenously, but when the same amount is given intraportally or intraperitoneally the splenectomized animals respond as well as controls Decreased antibody formation may be due to some extent to an absence of plasmacytes in the spleen and to the absence of the primary organ in which prolonged stasis of blood may occur

A pronounced leukocytosis usually follows splenectomy This increase in white blood cells in splenectomized animals may be due to the decreased activity of proteolytic enzymes which destroy lymphocytes and leukocytes in splenic sinusoids or in the hepatic portal vein Leukocytosis persisted for a month or more after splenectomy in rabbits and also after splenectomy combined with appendectomy [514] Leukocytosis following splenectomy has been established in rabbits, [48 10 6] man,[10 7] guinea pig [10 9] the albino rat [607] mice[974] and pigeon [514 10 5] Splenectomy produces a greater neutrophilic leukocytosis than trauma in man [369 607] Leukocytosis, following splenectomy in rabbits often doubles after *5 days* [1028] *The spleen,* therefore is considered to be the principal organ for the destruction of blood cells [10 6]

Splenectomy does not permanently alter the effects of some substances

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Effect on Erythrocytes

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range of 22 to 28 days to a range of 32 to 38 days during the month following splenectomy [368] A decrease in RNAase may also be a contributing factor to the increase in reticulocytes which has been reported in patients following splenectomy,[66] and to the immediate increase in thrombocytes [334]

A second possible effect of decreased proteolysis is the presence of the Feulgen *positive Howell Jolly bodies These bodies, which are considered* to be nuclear fragments in mature erythrocytes, occur in blood of splenectomized children and rats [368] Increases in Howell Jolly bodies were observed in 5 splenectomized adults [87] The presence of DNA in erythrocytes suggests the possibility of the absence, or a decreased amount of either an enzyme or a coenzyme in the hydrolysis of DNA in splenectomized animals, or the failure of retention and prolonged contact with the proteolytic enzyme

Splenectomy causes a temporary increase in erythrocytes in the blood, but anemia has also been reported to be one of the permanent effects An increase in erythrocytes and hemoglobin occurred in 5 patients after splenectomy [87] Polycythemia *was reported in dogs after splenectomy,*[775] but Chantel[154] found a transitory decrease Increased red counts in splenectomized animals and man are rare [368] Princigalli[81] reported a reduction in erythrocytes in only 3 out of 12 splenectomized dogs

One effect of splenectomy which would probably be attributed to its *function as a reservoir for blood, rather than to its proteolytic* function is the failure of splenectomized dogs to have a lower erythrocyte count after activity Rest produced a decrease in erythrocytes from about 6,000,000 to 4,320 000 per cu mm, but a decrease in erythrocytes did not occur in splenectomized dogs

Anemia has been reported to be *one effect* of splenectomy in rabbits, dogs monkeys[467] and white rats [349, 967] This anemia was related to the spleen's having a function in stimulating erythropoiesis, but may also be due to the decreased retention of iron in the reticuloendothelial cells of the spleen Products of the disintegration of erythrocytes[753] and other sub*stances produced* in the spleen[368] have been considered a cause of the anemia following splenectomy Injection of splenic extract has been reported to correct the disturbance in erythropoiesis [248] Splenectomy has also been used as a means of therapy in pernicious anemia [1142]

The erythrocytes were abnormally low for a month following splenec*tomy* in rabbits On the other hand a temporary rise in erythrocytes for 2 weeks occurred in one splenectomized rabbit [514] These differences may be due to individual variations, as reported by Princigalli,[81] or to a constant effect of the spleen upon decreasing the number of red cells [596, 833, 96]

10

Bone Marrow

Lymphocytes and plasmacytes are abundant in bone marrow, where these cells have the function of synthesizing and storing nucleoproteins for use by hematopoietic cells In the bone marrow, the formation and destruction of lymphocytes and plasmacytes maintains a balance between inter and intracellular proteins as it does in the spleen In the bone marrow of mammals, two additional nucleoprotein relations include (1) the release of blood platelets which contain ribonucleic acid (RNA) and (2) the disintegration of nuclei during the process of erythropoiesis Megakaryocytes and several other cells of the myelopoietic and erythropoietic series have a high content of cytoplasmic RNA, as do the plasmacytes but most of these cells differentiate into cells which contain specialized products such as hemoglobin in erythrocytes, or peroxidase granules in neutrophils

The bone marrow of mammals contains a considerable amount of lymphoid tissue Lymphocytes in the bone marrow have been considered by a few investigators to be a source of erythrocytes monocytes, plasmatocytes and fibroblasts [1106] However these proposed functions have not been established Disintegration of lymphocytes in bone marrow releases nucleic and amino acids, enzymes and other substances for hematopoiesis For example, amino acids in histone of lymphocytes are available for the synthesis of globin in erythropoiesis Another possible function of lymphocytes in hematopoietic tissues is that they are a source of the nucleoproteins which are important in stimulating myelopoiesis as Wright pointed out [935]

Most somatic cells do not have growth activity comparable to that of the cells in bone marrow The replacement of erythrocytes, alone occurs at an interval of a few weeks and runs into daily formation of thousands of millions of the red cells Quantitatively the amount of lymphoid tissue in bone marrow is as great or greater than that in the spleen of normal adult mammals In man, the amount of bone marrow (1000 to 3700 g) is said to be 3 4 to 5 9 per cent of the body weight [1103] and has been estimated by Doan (1931) to be about 1400 ml which is approximately the weight and volume of the liver [103]

LYMPHOCYTES AND LYMPHOID TISSUE

Lymphocytes, which compose by far the major part of the lymphoid nodules were present in the bone marrow of 39 per cent of 23 normal cases whose deaths were due to traumatism[1097] In these conditions the lymph nodes were considered to be normal rather than indicative of local inflammations[1097] Weiss[1090] states that aggregates of small and large lymphocytes occur throughout the bone marrow Yoffey[11 6] recognizes that bone marrow is subject to variation, and states that lymphoid nodules occur in one out of three red bone marrows of adult man However Lubitz and co workers[6 1] reported 50 instances of lymphoid nodules in tissue sections of marrow obtained by sternal aspirations They followed some of these patients and stated that lymphoid aggregates in the marrow were the earliest indication of lymphocytic leukemia

Lymphocytes are scattered singly throughout the bone marrow and have been considered to represent 5 to 15 per cent of the cells in differential cell counts of bone marrow compared with 20 to 40 per cent of the cells in differential counts of peripheral blood[35] It has been reported that lymphocytes represent 4 to 20 per cent of the cells in the marrow of 1 year old children[36] Holmes and Brown (1933) found that lymphoid cells represented 24 9 per cent of the cells in the marrow of 7 normal adults who had normal, peripheral differential counts[365] Leitner[597] summarizes the normal myelograms reported by 20 authors and finds that the percentage of lymphocytes varied from 2 to 38 Blisten (1944) calculated that there are 15 200 per cu mm lymphocytes in bone marrow compared with 3000 per cu mm in blood[1126]

Most hematologists report that lymphocytes comprise about 10 per cent of the nucleated cells in bone marrow and that the marrow represents *one of the largest collections of lymphocytes in the body* ' if these percentages are accurate[11 6] Some of these differences in lymphocytic ratios may be related to the activity of the particular marrow sample Mechanik (1926) believes that, normally only about half of the bone marrow is active at one time[1103] whereas Tullis[103] believes that only one fifth of the marrow is active at one time and that the remaining marrow forms an inactive reserve The differences in opinions concerning the presence or absence of lymph nodes in bone marrow may be related to the degree of activity or inactivity of the particular region of marrow studied For example, lymphoid nodules and a higher percentage of plasmacytes might occur in inactive regions of bone marrow and in this site these cells would represent a ' buildup" of protein storage or a ' bridging of the protein gap,' for use during active states of this particular region of the marrow

The percentage of lymphocytes in counts of cells in the spleen are about

twice as high as those usually given for bone marrow. For example typical lymphocytes are reported to represent 50 to 60 per cent of the cells in normal spleens.[36] This percentage would be expected to fluctuate considerably depending upon the relative amounts of red and white pulp used in determining the splenogram.

Increased numbers of lymphocytes in several forms of arrested maturation in erythropoietic and myelopoietic processes lend additional credence to the possibility that lymphocytes and plasmacytes store nucleoproteins for use by other cells. An evaluation of the literature on the amount of lymphoid tissue in bone marrow in pernicious anemia indicated extreme increases.[2, 1] The increase in the number of lymphocytes in pernicious anemia was so great that it was described by Custer (1935) and Rosenthal (1938) as giving the marrow a pseudofollicular appearance.[11, 7]

The effects of physiological and pathological conditions and of many experimental procedures on bone marrow cannot be correctly evaluated unless effects on the spleen are considered because there are functional interrelations between these two organs. One common factor in the spleen, bone marrow and lymph nodes is the presence of reticuloendothelial tissue, particularly the sinusoidal littoral cells. Physiological, pathological and experimental conditions which alter this system would be expected to have both direct and indirect effects upon cells in the spleen. For example there are differences in species of mammals in the relative amounts of red and yellow marrow which determine relations between the bone marrow and spleen. In addition it has been suggested that the spleen stimulates hematopoiesis.

Extramedullary hematopoiesis develops in the spleen in many mammals and in pregnant hamsters after the 9th day postcoitus.[18] The long bones of mice[300] and hamsters [35] contain only red marrow. Therefore, the spleen more readily becomes a hematopoietic organ than in those animals in whom only a small portion of the marrow cavity contains red marrow and in whom increased hematopoiesis can extend to the yellow, or inactive marrow. Granulopoiesis and erythropoiesis do not occur normally in the spleen of adult hamsters in contrast with the frequent presence of small hematopoietic foci in the spleen of some older mice of several strains.

The function of lymphocytes as protein synthesizing and storing cells may explain the increase of these cells in bone marrow in the earlier stages of several forms of maturation arrest of erythrocytes and granulocytes. Systemic protein anabolism may be normal but one cause of the maturation arrest is deficient. An essential component, enzyme or coenzyme which is essential for the development of hemoglobin or peroxidase is sometimes absent. One would expect to find an increased amount of protein stored in the bone marrow in these conditions. The increased formation of

lymphocytes in the bone marrow in these conditions would represent increased intracellular storage of nucleoproteins

The number of lymphocytes and the amount of lymphoid tissue in bone marrow might be increased *greatly during the earlier stages of conditions* of maturation arrest, but protein thus stored would be decreased in later stages, if systemic protein anabolism was decreased greatly However, acute posthemorrhagic anemias do not give a clear cut differentiation between the protein condition of erythrocytes and granulocytes, and the presence or absence of other agents necessary for maturation For example, deficiency under these conditions is a relatively more important factor in maturation of erythrocytes than in protein anabolism Leitner[597] reviews the literature on the significance of iron deficiency in anemia following hemorrhage

SINUSOIDS AND PLASMACYTOGENESIS

Sinusoids retain blood and thereby facilitate the retention of protein in the bone marrow by the formation of plasmacytes A characteristic feature of bone marrow, as well as of the spleen is the presence of sinusoids that have highly permeable and irregularly arranged reticuloendothelial cells, which afford a structural system to increase a phagocytosis [16, 393, 493] and exudation The fact that apparently no one has reported the presence of lymphatics in bone marrow[16, 251, 493, 661, 1080, 11 7] may be attributed to the presence of highly permeable sinusoids which fulfill the functions of lymphatics in bone marrow as in other tissues, such as the splenic pulp and the liver lobule [51, 1080, 1127]

The bone marrow sinusoid is usually described as a specialized, dilated region of a capillary derived from a terminal branch of a nutrient artery, and connecting with a venous capillary [16, 493, 601, 746, 886, 1080] However, the manner in which the arteries and sinusoids are connected is not so well understood as it should be [661] The "exquisitely thin"[746] wall of the bone marrow sinusoid is essentially formed by reticuloendothelial cells [393]

Some investigators believe that the wall of a distended bone marrow sinusoid represents a sieve like or latticed structure [51, 393, 493] Others believe that the wall is quite free of any openings which might function as mural stomata [493] *Nevertheless newly formed red blood cells and granulo*cytes freely, if not mysteriously pass through the sinusal wall into the blood within the sinusoids [493, 746] The lining of these sinusoids is often referred to as "delicate endothelium,"[493, 886] but it is composed of littoral, flattened and phagocytic reticuloendothelial cells which may detach themselves, roundup and become free macrophages in sinusoidal blood,[16] and may invade the loo e fluid stroma of the marrow [661, 746] A form of cyclic activity apparently prevails among the sinusoids, because sinusoids are

distended whereas certain capillaries in a capillary network are collapsed As capillaries dilate to form sinusoids others constrict to obliterate the sinusoid by preventing the passage of blood [858, 1079] Stasis of blood is conducive to plasmacytogenesis in bone marrow as well as in splenic sinuses and hemolymph nodes

Plasmacytes represent about 1 per cent of the cells which occur in normal bone marrow [735] The plasmacytes in bone marrow have been recognized to function in the formation of immune globulins and other proteins [23] Plasmacytes which do not normally occur in circulating blood were found by Markoff (1937) to increase in number in the bone marrow of a case of serum sickness but the day before the plasmacytes appeared in the marrow they had been observed in the blood smears [97] Leitner[597] points out that plasma cells increase in many but not all cases of serum hyperglobinemia Plasmacytes form extremes of 0 0 to 2 0 per cent (average 0 4) of the nucleated cells in normal bone marrow [1103]

NUCLEI OF ERYTHROCYTES

The nuclei which are lost during the blast stages of mammalian erythrocytes may contribute to the synthesis of several proteins Disintegration of the nucleus during globin formation is interesting because most of the amino acids which occur in globin of erythrocytes occur in histone of nuclei [1034] The erythrocytes of all animals lower in the vertebrate scale than mammals apparently retain the nucleus until cytolysis of the cell In the early fetal stage of man and presumably of other mammals most of the circulating red cells (including those within the vessels) are nucleated and have basophilic cytoplasm The percentage of nucleated cells decreases progressively as the embryo develops and as differentiation and specialization of structures and organs proceed The disposition of the nucleus of mammalian normoblasts presents another instance of uncertainty as to the recipients of its component trephones However these trephones probably are chiefly concerned in myelogenous hematopoiesis because the marrow is commonly the site of loss of the normoblasts nuclei

Most writers simply refer to the process by which red cell nuclei are lost as extrusion disintegration or dissolution but Cooke[183] discounts the idea of nuclear extrusion by stating that if it does occur it 'must be the unique example of cellular hara kiri in Biology Blackfan and coworkers[84] state that the pycnotic nucleus is extruded either as a whole or in fragments and that after extrusion of the nucleus the cytoplasm of the erythrocyte is faintly basophilic and filled with hemoglobin the cell is then called a polychromatocyte or reticulocyte

Leitner[597] apparently favors the view of Habelmann (1940) who be-

lieves that nuclei undergo disintegration and dissolution by proteolytic processes instead of being extruded This explanation is supported by Weiss[1079] Cooke[185] states that in human normoblasts the nucleus becomes pycnotic and karyolytic, with fusion of the chromatin particles to form a dense, structureless, nuclear mass which undergoes solution so that the young erythrocyte normally enters the blood without a trace of chromatin The presence of reticula, Howell Jolly bodies and Cabot's rings within reticulocytes, and the fact that basophilia soon fades when it is present in erythrocytes,[393] indicate incomplete enzymic action rather than nuclear extrusion

MATURATION OF ERYTHROCYTES

Blackfan and co workers[84] believe, along with Sabin and certain other polyphyletists, that the earliest or most primitive cell stage of maturation of the adult erythrocyte is an endothelial (reticuloendothelial) cell in bone marrow Thus, the consecutive cell stages given by these authors are (1) primitive cell (which arises from reticular endothelium), (2) megaloblasts (3) early eyrthroblast (4) late erythroblast, (5) normoblast, and (6) the reticulocyte or polychromatocyte which is non nucleated and (by further cytoplasmic changes) becomes the adult erythrocyte, or normocyte

It is extremely difficult to explain the stages involved in the process of deoxyribonucleic acid (DNA) and RNA changes, and the utilization of these nucleic acids or their components in the formation of *hemoglobin* with the essential albeit confusing terminology of the stages in maturation of erythroctyes Consideration of a few points in the cytology of denucleated reticulocytes which Kracke[559] believes "are intermediate stages between nucleated and non nucleated erythrocytes" will indicate the trephocytic nature of these cells with regard to DNA and RNA trephones Whether or not the basophilic strands that occur in polychromatocytes or reticulocytes contain DNA positive material has not been established However Kracke insists that it is not fully understood how reticulocytes lose the nucleus and acquire a mitochondrial network

Leitner[597] points out that the reticulum of reticulocytes is rich in if not composed of RNA and that Cabot s rings are unusual forms of Howell Jolly bodies hence they are nuclear remnants Cooke[185] believes that the Howell Jolly bodies are nuclear fragments which have broken off after the chromatin has aggregated into a structureless mass

It has been shown that cytoplasmic basophilia is very faint when it first appears in the earliest primitive red cells, increases throughout the primitive stage, reaches its maximum density in the megaloblasts and begins to decline in very late megaloblasts or earliest erythroblasts At the same time, the nucleus becomes increasingly *pycnotic and karyor*

thetic[84] Then hemoglobin appears in the very late megaloblasts or first early erythroblasts which occur when the nuclei become moribund and cytoplasmic basophilia declines As the clumps of nuclear material and basophilia decrease the amount of hemoglobulin steadily increases until it reaches its maximum in the earliest reticulocytes

The appearance of hemoglobin in the cytoplasm is concurrent with the onset of the destruction of the nucleus and the depletion of basophilia It increases in amount as these destructive processes continue This process indicates the possibility that the DNA of the nucleus and the RNA of the basophilic cytoplasm are being used in the formation of hemoglobin Thus, it would also appear that the nucleus is destroyed by enzymic action rather than by being fragmented or entirely extruded

It is generally conceded that the nucleated mammalian red blood cell usually loses its nucleus before entering the circulation but that nuclear substance may persist for a short time in circulating erythrocytes of normal young (6 per cent) and adult (0 1 to 1 per cent) individuals[1103] An increase in the number of these young erythrocytes commonly designated by the ineptly chosen name of reticulocytes, in the blood is the surest index of accelerated hematopoiesis[1103] Leitner[597] believes that polychromatophilic erythrocytes are derived from the marrow reticulum cell by five successive steps proerythroblast early basophilic normoblast polychromatic normoblast and orthochromatic normoblast in which the spheroidal and solid nucleus loosens to form the network characteristic of the typical reticulocyte Usually most investigators are content to accept the origin of the reticulocyte as being from the normoblast and consider this cell to be an erythrocyte when its RNA reticulum becomes obscure and disappears from view in routinely stained blood film preparations

The non nucleated erythrocytes of mice contain an appreciable amount of nucleic acid but it is difficult to determine from the results of Whitfeld's[1090] work whether the major part of the nucleic acids were in the red cells or in the blood plasma because he used hemolyzed red cells in plasma However he arrived at the conclusion that the solid residue obtained by hemolyzing blood of mice that had 25 per cent of the red cells infected with *Plasmodium berghei* contained 12 times as much DNA and 20 to 25 times as much RNA as the residue from the blood of uninfected mice This work indicates that the mature, non nucleated mammalian erythrocyte probably contains elements capable of providing raw material for the synthesis of DNA and RNA in this species of malarial parasite

Warburg (1914) showed that the rate of respiration in red blood cells is related to the amount of cytoplasmic basophilia rather than to the

presence of a nucleus, as his earlier work seemed to indicate[104] Brachet[104] states that Warburg's finding that nucleated red blood cells of mammals (reticulocytes) have a higher rate of respiration than the adult mammalian red cell has been verified by Caspersson and P Dustin Since metabolic activity is related to respiratory activity, it is reasonably safe to assume that the nucleated red blood cell contains more trephones than the enucleated red cell It may also be assumed that most of the trephones lost with the nucleus of red cells are utilized by other cells rather than being destroyed

Nucleated erythrocytes of birds contain chromatin consisting of a "salt of nucleic acid' with histone as its protein base, "as in the thymus of the calf or in echinoderm spermatozoa"[653] However, the nucleated red cells of birds and lower vertebrates are said to contain relatively little protein[807] Ham[393] and Jordan[493] indicate that the mother cells of mammalian erythrocytes are probably well supplied with DNA in the nucleus, and RNA in the cytoplasm

MEGAKARYOCYTES

The high content of cytoplasmic RNA, the absence of mitosis and the pinching off of cytoplasmic particles which are considered to be blood platelets, are characteristics which indicate that the megakaryocyte may function in protein synthesis and storage The megakaryocyte is one of several kinds of giant cells which occur in bone marrow[834] Other giant cells in marrow include osteoblasts and multinucleated plasmacytes[834] Extramedullary foreign body giant cells occur in various pathological conditions in man and are believed to have multiple origins, but probably arise chiefly from reticuloendothelial cells[141] Some investigators believe that foreign body giant cells in infectious granulomas form by the fusion of monocytes[314] or endothelial leukocytes by mitosis without division of the cytoplasm,[314, 713] and from the fusion of plasmacytes[834] Megakaryocytes sometimes phagocytize various blood cells such as erythrocytes[4 0] neutrophils[659] eosinophils[4 0] plasmacytes and lymphocytes[535]

Megakaryocytes can phagocytize other cells, but their significance in phagocytosis has not been established Kuyts (1931) suggests that the apparent erythrophagocytosis by megakaryocytes is an artifact *due to* superposition[75] However, 4 μ sections of megakaryocytes in bone marrow of hamsters, prepared by the celloidin paraffine method of Crabb[196] indicate that this cell does phagocytize erythrocytes platelets and polymorphonuclear leukocytes[535]

The functions of the megakaryocyte are partly related to its great size (35 to 40 μ)[1103] which is a physical characteristic enabling it to ingest cells of various sizes Apparently megakaryocytes function as

trephocytes chiefly by phagocytosis and the digestion of cells or substances, and dispensing the trephones thus obtained in the form of blood platelets

The cytoplasm of most of the stages of megakaryocytes has a high content of RNA Use of ribonuclease (RNAase), followed by staining with methylene blue, and the method of staining marrow with methylene blue at pH 4 9 indicate that megakaryocytes contain greater amounts of PNA than that which occurs in the cytoplasm of most cells These methods show that most stages of megakaryocytes contain about the same amount of cytoplasmic RNA as plasmacytes[535] However, Wislocki and co workers[1107] believe that the RNA, as indicated by basophilia in the cyto plasm of megakaryocytes, is moderate in amount Other substances, such as mucoprotein or glycoprotein,[310] glycogen[1067] and peroxidase[5 1] also occur in the cytoplasm of megakaryocytes The high concentration of RNA may indicate that this cell is important in the synthesis and storage of protein and in the release of protein in platelets The significance of RNA in the cytoplasm of platelets has been considered by several investi gators[3]

Platelets which arise from megakaryocytes according to the theory proposed by Wright,[75] represent cytoplasmic particles which are controlled by several normal physiological factors and are altered by certain patho logical conditions The platelets of mammals therefore were considered by Wright to be detached cytopla mic particles free in blood[1103] However, studies by Voit and Kempa (1934) indicate that Feulgen positive material may be included[1107] A comparison of several histochemical tests on platelets and megakaryocytes indicates that platelets probably arise from megakaryocytes[1107] Systems of canaliculi occur in megakaryocytes during the process of platelet formation[75] Electron micro copic studies have demonstrated that vesicles with a diameter of 100 Å form in the cytoplasm of megakaryocytes during the early stages of platelet formation[11] Cer tain investigators believe that the formation of platelets represents a termi nal disintegrative process of megakaryocytes rather than a normal func tion because 15 to 20 out of 100 megakaryocytes (15 to 20 per cent) have been reported to be degenerating[75]

11

Intestinal Lymphoid Tissue Relationships

A unique relation between alimentary protein and the method of its utilization is afforded by the presence and arrangement of lymphocytes and *plasmacytes in the mucosa of the intestines, where these cells are* interposed between the lumen of the intestine and the hepatic portal vein Small lymphocytes and plasmacytes from the intestine release nucleoproteins by disintegrating in the mucosa, hepatic portal vein and hepatic sinusoids In addition small lymphocytes which pass through the liver into the general circulation provide systemic distribution of a certain amount of nucleoproteins by their disintegration in various tissues and organs

Intestinal lymphoid structures occur as discretely delimited areas which are dispersed throughout the length of the intestine Intestinal lymphoid structures are characterized by the absence of well defined afferent lymphatics and include the Peyer s patches, solitary follicles, cecal lymphoid patches and in certain forms, the vermiform appendix Intestinal lymphoid tissues are primarily composed of lymphocytes

Large lymphocytes in the germinal centers of the intestinal lymphoid structures can use or store amino acids directly as they pass through the lymphoepithelium into the lymphoid tissue Most cells produced by mitosis of large lymphocytes become small lymphocytes which do not divide Therefore, small lymphocytes do not utilize the contained nucleic acids for proliferation of their kind but transport this protein, chiefly as histone and deoxyribonucleic acid (DNA) through the hepatic portal veins or the lymphatics into the systemic circulation Small lymphocytes that enter the lymphatics in the tunica propria of the intestine may remain in the pancreas Aselli or other mesenteric lymph nodes for some time, where ome of them transform into plasmacytes before entering the blood stream Those entering the venous system pass directly over the portal vein to the liver where ome of them may transform into ribonucleic acid (RNA) bearing plasmacytes and release their cytoplasmic RNA for use by the liver

It should be pointed out at this time that lymphocytes and plasmacytes are not indispensable for protein assimilation by other cells Instead, they represent an accessory storage cell

In evaluating the significance of lymphocytes and plasmacytes in protein metabolism, it is necessary to recognize that a very slight increase of RNA and protein within numerous cells represents the first or earlier stages of protein storage For example after a protein low diet or total caloric restriction, realimentation causes a slight increase of RNA and protein in various tissues and organs such as the liver and salivary glands The increase of lymphoid tissue follows a somewhat longer interval

The idea that protein is stored in the blood is comparatively new Bach[3] states that until 1935 physiologists believed that there was very little ' replacement of the protein of living tissue by dietary amino acids and that this was merely "to compensate Brobeck[112] points out that the body of a normal well fed adult contains 2 to 3 kg of reserve protein He cites work indicating that hypophyseal growth hormone facilitates nitrogen retention and protein synthesis, whereas adrenocortical extract increases urinary excretion of nitrogen and potassium Sprinson and Rittenbert (1949) concluded, from experiments with isotopes that the volume of pooled free amino acids in the human body represents 0 4 g nitrogen per kg of body weight [3] Most writers believe that the amino acids which enter the body are carried by the blood to all tissues where they may be utilized or incorporated [41] However those amino acids which pass through the lymphoepithelium (covering of intestinal lymphoid structures) may be directly and immediately utilized by large lymphocytes in the intestinal lymphoid tissues in the synthesis of DNA and histones These nucleoproteins are retained chiefly in the nuclei of small lymphocytes and may be used in the formation of plasmacytes a process which incorporates significant quantities of dietary amino acids directly from the lumen of the intestine Thus by virtue of this interposed location the numerous lymphoid structures and aggregated cells in the mucosa and tunica propria of the intestine have direct access to dietary amino acids and other substances required for the synthesis of nucleoproteins This interposed location is very significant in view of the great number of lymphocytes and plasmacytes contained in the numerous and various intestinal lymphoid structures in addition to the free and aggregated cells in the mucous membrane and tunica propria Lymphocytes and plasmacytes in the tunica propria of the intestine may supply trephones for transportation by the hepatic portal venous tributaries or by the lymphatic system via the lacteals which pass to the mesenteric lymph nodes In turn trephones can enter the blood vessels that arise in mesenteric lymph nodes and pass to the liver However, the obvious and more important route for the passage

of intact small lymphocytes and trephones, freed from lymphocytes and plasmacytes in these nodes, is via the lymphatics into the thoracic lymph duct from which these cells and/or their components enter the blood vascular system

FUNCTIONS

The functions which have been assigned to lymphoid tissue in the digestive tract are as numerous and variable in scope as those functions ascribed to this tissue in other regions of the body Drinker and Yoffey[2] cite results which indicate that the tonsils, lymphoid tissue in the naso pharynx and digestive tract were popularly supposed to be a defense or barrier mechanism The theory of the defensive function of lymphoid tissue has recently been amplified and extended by various investigators to include the filtration of noxious material out of the circulation, and antibody formation in lymph nodes [1] Lymph nodes in normal healthy animals were found to contain bacteria, because the nodes are constantly filtering out particulate matter which may enter lymphatics In 1910, Adami elaborated this idea for the mucosa of the stomach and intestine He believed that these organs are constantly subjected to a process of "subinfection " [51] After reviewing the literature on the theory of intestinal bacteria as it is applied to the function of lymphoid tissue in health, Drinker and Yoffey [1] concluded that most of the work was speculative, that experimental proof was difficult to obtain, and that the mere presence of bacteria in an organ or structure does not mean that it is not discharging its normal functions The idea that the function of intestinal lymphoid tissue is primarily if not wholly, defensive has resulted in a number of papers which extend over a period of several years [1]

A well established and very important function of intestinal lymphoid tissue is that of lymphocytopoiesis In many lower vertebrates, such as the cyclostomes (hagfish) and certain higher forms the intestine is probably the first, certainly the most important structure to form blood cells and lymphoid tissue [536] Intestinal lymphoid tissue is regarded as an important source of lymphocytes which emigrate to other organs These cells also have been considered stem cells capable of producing erythroblasts and other blood cells [493]

Lymphoid tissue and lymphocytes have been assigned many functions in nutrition These cells were believed by earlier investigators to aid in protein and fat transportation, and in absorption and assimilation [898] The rapid decrease of lymphoid tissue in hungry, and the increase which follows the administration of favorable diets show an interrelationship between ingested food and its assimilation [9 2 1 898]

It has been shown that intestinal lymphoid tissue plays an important

part in gastric and intestinal biosynthesis, and in the availability of vitamins of the B complex.[2, 501, 1137] A number of investigators have pointed out that lymphoid tissue is dependent upon the B vitamins for growth and functional ability and that this tissue atrophies in B deficient animals.[214, 439] Hunger,[9, 51] or chemicals such as arsenicals,[59] benzene,[514] cortisone[96] and others destroy lymphoid tissue throughout the intestinal mucosa without showing a selective effect on any one form of lymphoid structure such as solitary follicles or lymphoid tissue in any one area. Obviously, these results are the result of systemic effect.

Most herbivores have a higher percentage of lymphocytes than granulocytes but this relation is reversed in carnivores. The predominance of granulocytes over lymphocytes in carnivorous animals may be important as a source of peroxidase for relatively greater amounts of protein catabolism,[5] whereas the greater percentage of lymphocytes in herbivores may be indicative of the function of lymphocytes in nucleoprotein synthesis and as a protein storage the transportation cell. Brilla[110] states that the white blood cells in herbivorous mammals average about 65.20 per cent lymphocytes and 30.47 per cent granulocytes, whereas most carnivores have 40 to 50 per cent lymphocytes and about 57 per cent granulocytes in the peripheral blood. Cats have 59.5 per cent neutrophils and 5.4 per cent eosinophils with only 31 per cent lymphocytes and 4 per cent monocytes. Dogs have 68 per cent neutrophils and 5.1 per cent eosinophils with only 21 per cent lymphocytes and 5.2 per cent monocytes. Rabbits have about 48 per cent granulocytes, 39 per cent lymphocytes and 8 per cent monocytes.[3]

Peyer's Patches and Solitary Follicles

Peyer's patches are scattered throughout the length of the small intestines—and scattered typical solitary lymph nodules occur in the mucosa of the mouth, esophagus, stomach, small and large intestine.[91] The significance of the solitary nodules is difficult to evaluate because usually they are not readily visible until the intestine has been fixed and cleared. They cannot, therefore, be surgically extirpated. Furthermore, the slight variations that follow experimental procedures with intestinal lymphoid tissues are difficult to evaluate.

A review of the literature on Peyer's patches shows that there is a general conformity in localization and distribution of these structures in mammals.[51] Peyer's patches are essentially aggregates of solitary nodules.[1060] Three to 24 of them occur as lymphoid tissue in the mucosa of the small intestine of mammals. Peyer's patches are devoid of true afferent lympatics like other intestinal lymphoid tissue. Consequently collecting sinuses are absent. However, as shown for the intestinal lymph

oid tissue in the appendix of the rabbit, capillaries pass from the lumen of the appendix (via the flask shaped mucous glands) through the lympho epithelium and continue through the parenchyma of the nodule as discrete tributaries of the internodular collecting lymphatics [07]

The number of Peyer's patches[535, 1060] varies in species, strains and individuals For example, counts of Peyer's patches in 780 mice belonging to five strains indicated that the lowest range occurred in C57 Black, which had an average of 6 3 patches for 249 intestines The highest average number of Peyer's patches was 10 7 for 124 C3H mice [517] The average number of Peyer's patches in the small intestine of 46 rabbits (chiefly New Zealand white) was 5 5, with no significant sex difference in size or weight of the patches [650] Peyer's patches in the small intestine of 400 normal hamsters ranged from 5 to 18 in number with an average of 9 95 [516] The mean and frequency distributions were essentially the same as in mice [517] Hamsters with a mean of approximately 10 patches have more than the usual number of 6 found in rabbits[705] or the average of 6 3 found in C57 Black mice [517] but considerably less than the range of 18 to 26 for rats[456] and 20 to 30 for man [450]

No significant sex difference was found in the number of patches in normal hamsters The 200 males had a mean of 10 1, and the 200 females, 9 8 The frequency distributions were also the same for both sexes according to the chi square test for goodness of fit [797] Solitary follicles in the small intestine of hamsters were much larger than a pin point, but were found in only a few of the 400 control animals The maximum number of solitary follicles found in a single small intestine was 7 None of the other 399 control hamsters contained more than 4 Thus, the Peyer's patches comprise almost the total volume of the organized lymphoid tissue in the small intestine of hamsters, but each villus contains many lymphocytes and plasmacytes [535]

Cecal Lymphoid Tissue

Small areas of lymphoid tissue occur in the walls and apex in the cecum of most rodents There are great differences however between rabbits (order Lagomorpha) and true rodents with regard to the cecal lymphoid tissue Ottaviani[76] noted that some of the lymphoid tissue in the intestine of the rabbit does not have homologues in other mammals and that the classification of these lymphoid structures in the rabbit is not applicable to that adopted for man Rabbits have conspicuous and specialized lymphoid structures near the ileocecal valve in addition to a very large vermiform appendix The sacculus rotundus connects the ileum with the cecum and forms a lymphoid antechamber to the ileocecal valve and cecum Structurally, the sacculus rotundus resembles the appendical and cecal lymphoid

patches much more than it does the Peyer s patches The sacculus rotundus does not occur in laboratory rodents It appears to be peculiar to the Lagomorpha, at least in North America and domestic forms Since the sacculus forms an enlarged specialized lymphoid antechamber of the cecum, and may be distended with chyle by temporary closure of the ileocecal valve it probably plays an important part in the absorption of the products thus retained

The sacculus rotundus may also function as a temporary reservoir for chyle and thus following closure of the ileocecal valve retard the passage of material through the large intestine and, thereby promote colonic digestion of cellulose and the absorption of nutrients, water and bile in cecectomized rabbits[4 3] This mechanism doubtless is very effective in normal intact rabbits

The cecal patches of lymphoid tissue are usually situated in the wall on opposite sides of the ileocecal valve in lagomorphs Cecal lymphoid tissue also includes the tonsilla ileocecalis major and minor when the minor is present Among the wild lagomorphs the pika has the tonsilla ileocecalis major and minor modified to form elongated tubular processes the lumina of which are in intimate connection with the lumen of the cecum so as to permit free interchange between the cecal and lymphoid tissue products

One to several smaller patches are present in the cecal wall in rodents such as mice rats hamsters and western fox squirrels The cecum of the vole Arvicola or Microtus[130] and guinea pig have multiple sacculations or compartments open to the lumen Each sacculation often has a large parietal lymphoid patch Contrary to the idea accepted by some histologists[91] the guinea pig s cecum does not have a vermiform appendix The lymphoid tissue in the cecum of all the domestic and wild rodents examined by us was limited to aggregates of nodules which were similar in structure to Peyer s patches but no structure resembling the spiral valve or the vermiform process of lagomorphs was found[397 0 04]

The patches of lymphoid tissue in the cecum of rodents is the Peyer s type the most massive of which is usually situated in the blunt apex of the rather simple sack like cecum Only a few of 300 mice of five inbred strains which included C57 Black C3H Swiss and Dba line 212 had an extra lymphoid patch in the cecum but this was the only clear cut variation found[517]

Cecal Form and Feeding Habits

There is a fairly consistent correlation between the structure of the digestive tract and the natural food and feeding habits of animals[759] for example the ab ence length and morphology of the cecum and

colon [219 55 6 9 730] However, as Gadow pointed out for birds[759] and Oppel[759] for all classes of vertebrates, it is often difficult to relate the cecum to nutrition Also, data on the occurrence of cecal lymphoid tissue is often scanty or absent

Gallinaceous birds, such as chickens, turkeys and partridges, are pri marily seed eating forms and have long, paired ceca Predatory birds commonly have short, paired ceca, and others may have long (8 to 9 cm), paired ceca [730] Pigeons, which are primarily seed eaters, and certain sparrows and finches, which eat both seeds and insects, have short, paired ceca Some of the woodpeckers, which are primarily insectivorous and, in season, frugivorous, have no cecum at all [730] The presence of a capacious, elaborate cecum, as in certain herbivores, or the absence of a cecum in both birds and mammals, as in certain carnivores and insectivores, indicates that this structure is endowed with special functions related to diet How ever, the work of many investigators suggests that practically all available carbohydrate, fat and protein have been absorbed before the contents of the small intestine pass through the ileocecal valve [8 0]

A certain amount of biosynthesis of B vitamins occurs in the first stom ach of ruminants and in other regions of the intestine in various animals However, the cecum in most non ruminants is by far the most favorable region of the gastrointestine for the synthesis of vitamins and the digestion of cellulose by organisms However, this efficiency of the cecum and colon (and the rumen) varies in different animals because it is related to multiple factors, the most obvious of which is size or capacity The amount of lymphoid tissue in the cecum and appendix is primarily related (1) to the capacity of the cecum (2) to the kind of material and length of time it is retained and (3) to the extent of bacterial and protozoal activities within this organ

Most carnivorous mammals, such as domestic cats, have a short cecum with an apical, lymphoid cap or patch Some carnivorous mammals and birds do not have a cecum Mammals which do not have a cecum include the long tailed shrew a cannibalistic insectivore,[204] the European mole (Talpa) and the bat (Vesperugo) insectivores the dormouse (Myoxus), a rodent, and the sloth (Bradypus), and edentate [730]

Omnivores usually have a relatively larger and more capacious cecum than carnivores, however the cecum of an adult man is about 6 0 cm long and 7 5 cm wide [335 450] and that of the orangutan is about the same size [730] Most dogs have a flexous pointed cecum which is 10 7 to 15 25 cm in length [730 943] Domestic dogs are classified as carnivores, but are capable of utilizing nearly 5 per cent of ingested cellulose, in contrast with man who can utilize so very little cellulose that it is of no nutritional signifi cance [412]

Monogastric herbivores, the cecum of which attains a relatively maximal size utilize about 25 per cent of the cellulose consumed Certain of the e animals especially the grazing mammals have a large capacious cecum the size of which does not appear to be definitely related to the type of stomach possessed by the animal That differences in the size of the cecum of ruminants and non ruminants is not greater is surprising since the first stomach (rumen) in ruminants serves as a fermentation pouch for the preliminary digestion of food, for the production of vitamin B_1 ,[1137] and for the synthesis of protein from urea by the biosynthetic activity of microorganisms within the rumen[2] Thus the specialized stomach and cecum of ruminants afford a highly efficient dural arrangement to insure an opportunity for maximal digestion and con equently maximal ab orption of food Ruminants (Pecora) have a large cecum in addition to four stomachs[730] but the cecum is hardly comparable in size to that of the hor e[730] and, probably other non ruminating grazing mammals The cecum of both types of grazing mammals has special functions of im pounding material to further the fermentation of the cellulose (perhaps *differential in effects) and probably of dead resident organisms and the* biosynthesis of certain members of the vitamin B group The cecum also acts as a special organ for absorbing the products of this fermentation as has been postulated for the cecum of the horse[1137] and rabbit[535] and for absorbing the synthesized vitamins Adult cattle have a flexed cecum which averages about 0 76 m[943] to 1 40 m[730] The cecum of sheep is about 0 35 m in length,[730] whereas that of the horse is about 1 22 m long and has a capacity of 26 5 to 30 38 l[943]

Members of the Lagomorpha probably have the most remarkably con structed and most efficient cecum of all mammals[197 04] A very large cecum occurs in rabbits and hares the most widely known members of this order The cecum of these animals is equipped with a spiral valve which makes about 25 turns and terminates in a well developed vermiform ap pendix[197 20] The pika or coney (*Ochotona princeps*) has a peculiarly sacculated procolon[513] between the cecum and colon as well as a true vermiform appendix However its cecum differs from that of the other lagomorphs in having more turns in the spiral valve Also the tonsilla ileocecalis major and minor are greatly extended to form tubular vermi form diverticula which are composed primarily of lymphoid tissue The pika subsists chiefly on dried grass 9 to 10 months of the year because this species lives at high altitudes near the timberline The cecum is wide and long and has a spiral valve like fold in the pika and the various kinds of rabbits and hares (Dutch New Zealand white Flemmish Belgian wild cottontail black tailed and white tailed jack and snowshoe) which we have examined The cecum in 300 embalmed adult New Zealand white

rabbits was 20 0 to 36 0 cm in length [53] The potential length of this organ in the rabbit is much greater than its outside dimensions would indicate because of its shelf like spiral valve which makes approximately 25 turns within the lumen A comparable provision occurs in the large cecum of the guinea pig which is divided into a number of stall like compartments by thin, wide, internal, transverse folds of the mucosa that form incomplete *circular partitions*

Correlation between the size and architecture (smooth, haustrated partitioned or provided with a spiral valve) of the cecum and the kind of food eaten by the animals is essentially the physical foundation for the explanation of the digestive and absorptive processes involved The adult rabbit by virtue of its very long digestive tract and highly specialized cecum and appendix, can maintain itself well on a diet that contains only 12 to 15 per cent protein A diet that contains 16 to 20 per cent protein is adequate for pregnant and lactating rabbits [895] Rats require 25 to 30 per cent protein in the diet for "optimum growth and excellent reproduction and lactation "[662] Apparently, very few other mammals can remain healthy and reproduce normally on a diet that contains even the maximal amount of protein required by some of the lagomorphs for optimal reproduction and growth This difference in the amount of required protein apparently is chiefly compensated by activities of the intestinal flora and the absorptive capacity of the specialized lymphoid tissue in the cecum and appendix

The unusually large and highly specialized cecoappendix of lagomorphs suggests important functions, some of which apparently are related to the hydrolysis of cellulose and to the biosynthesis of proteins and B vitamins However Herndon and Hove[4 3] found that surgical removal *of the cecum and appendix did not inhibit the digestion of* cellulose in 5 rabbits which survived this very severe operation up to 9 months About 15 of the 20 cecectomized rabbits died in surgical shock The daily average growth rate of the 5 cecectomized rabbits was 22 g, whereas the control rabbits averaged 25 to 30 g However the food supplied these cecectomized rabbits was well fortified with vitamins It included at least one (biotin) of the two vitamins (biotin and *para* aminobenzoic acid) which are required for cellulose digestion *in vitro* [7-] Also, the diet of these cecectomized rabbits apparently had a much higher protein content (25 per cent casein) than that of rabbits in nature or that required by domestic rabbits for optimal maintenance (12 to 15 per cent) and slightly exceeded the 16 to 20 per cent *protein that is recommended for pregnant and nursing* females [895] The high protein content of the diet that was fed to the cecectomized rabbits probably furthered cellulose digestion Hall (1954) has shown that 'partial hydrolysates of proteins stimulate the rate of cellu

lose digestion' [7] Nevertheless, utilization of sodium and potassium was greatly lowered and fat and protein digestion were reduced to a lesser extent However, calcium and ash utilization remained normal in the 5 cecectomized rabbits [4 3]

The conclusions of Herndon and Hove[4 3] that total cecectomy did not inhibit cellulose digestion in domestic rabbits does not militate against the possibility that the cecum and appendix form a special adaptation to insure the digestion of cellulose, and the synthesis and absorption of pro teins vitamins and other substances in wild rabbits living under conditions far less favorable than may be maintained in the laboratory

Absorption from the Cecum

The elaborately pecialized structures within the cecum in certain mam mals indicate that this organ at least in some animals is a specialized adaptation and plays a part in biosynthesis These ceca retain chyle and absorb certain materials from it Most of the herbivores (hor e guinea pig chinchilla) and some of the omnivores (swine) have sacculated, haustrated, partly partitioned or otherwise multiple compartmented ceca Some of the omnivorous (mouse hamster) and many of the carnivorous mammals (cat) have a nearly smooth walled cecum usually with a well defined patch of lymphoid tissue at the apex Lagomorphs have an in ternally projecting rather thin connective tissue supported fold of the mucosa forming a sort of piral valve, which makes about 25 (rabbit) or more (pika) spiral turns in the cecum [203 730] Notwithstanding the un usual structural specialization of the cecoappendix in rabbits it has been reported that cecectomy failed to prevent the normal digestion of cellulose in laboratory animals fed an optimal, high protein diet [4 3] Whether or not cecectomized rabbits can survive the rigors of a diet that consists chiefly of low protein roughage which is often encountered in natural conditions remains to be seen

Specialized villus like mucosal processes up to 11 mm in length and exhibiting intense alkaline phosphatase activity in the epithelium pro ject into the lumen of the cecum proper and the ampulla caecalis coli in the red tree mouse *Phenacomys longicaudus* [1061] Voge and Bern[1061] re viewed the literature on villi of the spiral valve in lagomorphs and state that Tullberg (1899) observed numerous long branched villi on the rim of the spiral valve of the cecum' in the Siberian pika, *Lagomys alpinus* (*Ochotona alpina*) but failed to describe in detail the number size or structure of these villi These investigators[1061] found villus like tructures similar to those ob erved by Tullberg in an alcohol preserved American pika *Ochontona princeps* which were present on the rim of the spiral valve only and were shorter wider and flatter than those of Phena

rabbits was 20 0 to 36 0 cm in length[535]. The potential length of this organ in the rabbit is much greater than its outside dimensions would indicate because of its shelf like spiral valve which makes approximately 25 turns within the lumen. A comparable provision occurs in the large cecum of the guinea pig which is divided into a number of stall like compartments by thin, wide, internal, transverse folds of the mucosa that form incomplete, circular partitions.

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live primarily on a plant diet that has a low protein and a high cellulose content, this practice is an adaptation whereby these animals may synthesize various proteins and utilizable forms of carbohydrates and conserve substances, such as vitamins, minerals and components of mucin The practice of coprophagy is so widespread among laboratory animals that prevention of reingestion of feces by animals being used in nutritional experiments is required

Rabbits have been suspected of chewing the cud or reingesting feces (pseudorumination)[1013] for many years[268, 63] However, it has developed that the rabbit is probably the only common laboratory mammal which circumvents the usual precautions for the prevention of coprophagy, by ingesting feces directly from its anus Eden[68] included 50 mg of copper sulfate in the diet of rabbits which were kept in individual cages that had a wire mesh bottom to permit fecal pellets to pass through He placed a wide wooden collar around the animals necks in order to prevent the ingestion of feces directly from the anus By this method, he found that rabbits normally ingest 54 to as much as 90 per cent of their feces Rabbits which were prevented from reingesting feces digested an average of only 63 per cent of the food eaten Following removal of the collar, they digested 77 per cent Kulwick and co workers[571] found that rabbits voided 29 per cent more fecal material (when considered on a dry basis) when they were prevented by a collar from eating feces Other investigators stanchioned rabbits to prevent ingestion of the feces while being voided[4 3] In addition Herndon and Hove[4 3] observed that cecectomy apparently disrupted the neural chemical or physical motivator which induces coprophagy in normal rabbits

Taylor[1013] points out that the soft, mucus coated small round pellets often seen in the cardia of the stomach have been reingested directly from the anus by the rabbit He thinks that these feces are ingested during early morning by tame rabbits but late in the day by wild rabbits We have observed intact pellets near the cardiac valve in the stomach of a New Zealand white rabbit and noted the progressive digestion of these pellets as they were moved into the fundus Thus, we observed intact pellets near the cardiac valve partly emptied to completely emptied or ghosts of mucus coverings in the pyloric half of the fundus The hard day pellets of rabbits are supposed to be derived from material which has remained for some time in the cecum The soft pellets, which are reingested are believed to be derived from material which has bypassed the cecum[219, 4 3] The niacin and riboflavin content of soft feces are believed to be 3 to 4 times that of the hard feces in rabbits[571]

Rabbits caged so as to prevent coprophagy, did not grow as rapidly as the group in which coprophagy did not grow as rapidly as the group in

comys" They state that this type of villus occurs in the rumen of cattle and sheep which are the only mammals that have villi comparable in length *to the cecal villi of Phenacomys*

Villi are usually limited to the small intestine of adult mammals[1061] Cecal villi, however, occur in the cecum proper, and in the ampulla caecalis coli, and extend into the first coil of the postcecal spiral in the red tree mouse, *Phenacomys longicaudus* The long (up to 11 mm) cecal villi contain numerous acinar glands which are absent in the intestinal villi of this mouse Villi were not found in the cecum of five other genera of the subfamily Microtinae to which Phenacomys belongs[1061] Voge and Bern[1061] point out that cecal villi have not been demonstrated in any of the rodents previously discussed in the literature The presence of numerous acinar glands suggests that the cecal villi in Phenacomys are more nearly related to mucosal plicae than to true intestinal villi, as is the case with the "villi" in the pyloric end of the duodenum of mice

Recorded similarities and differences between the ileal and cecal villi of Phenacomys include the presence of intense alkaline phosphatase activity in the epithelium of both types of villi which is especially important because carbohydrate transport mechanisms apparently require alkaline phosphatase[1061] Voge and Bern suggest that the similarity of cecal and ileal mucosae may be specially adapted to a diet generally restricted to pine needles the nutritive substances of which are obtained by the absorption of "products of bacterial disintegration "

The cecum of the duckbill (*Ornithorhynchus anatinus*),[730] mice, cats and many other mammals is usually characterized by the presence of a well developed lymphoid mass at the apex It is interesting to observe that this apical lymphoid patch also occurs in the cecum of the red tree mouse, turkey and chicken[535] More significant phylogenetic and ontogenetic evidence that the cecum has definitely absorptive functions is indicated in chickens which have paired ceca with basal portions that contain intestinal villi[131] These and villi in the rectum[131] and lymphoid elements in the cloaca probably have the special function of salvaging the hydrolyzed or synthesized food materials which escape absorption in the small intestine and ceca Villi are also present in the appendix of the fetal rabbit *and do not disappear until the end of the 2nd or during the 3rd* week after birth [0]

COPROPHAGY

Coprophagy is a normal practice of rabbits and rodents and is a natural source of increased amounts of B vitamins nucleic acids and mucopolysaccharides (MPS) which are derived from bacterial action and the disintegration of bacteria in the stomach and intestine In certain animals which

conclusions, drawn from the importance of bacteria in the rumen have been assumed to have a similar importance in the cecum of Lagomorpha

Hydrolysis of cellulose to short carbon chains is a source of utilizable carbohydrates and is indirectly important as a substrate for the intestinal flora and the synthesis of B vitamins and amino acids by bacteria Cellulose fermenting bacteria in the rumen convert cellulose into an absorbable form [19] such as glucose [644] In rabbits this hydrolysis normally occurs in the enormous cecum and to a lesser extent in the intestine [4 3] By the practice of coprophagy these shorter carbon chains are subjected to the action of enzymes in the stomach and first part of the small intestine Pentosans in herbivora are considered to be between 40 to 80 per cent utilizable [41]

Bacteria in the intestines and cecum synthesize various growth promoting factors for the animal and for the proliferation of other bacteria Peterson and Peterson[800] list many of these bacteria which synthesize B vitamins antihemorrhagic compounds and uracil The obvious relation between the size and structure of the cecum and the lymphoid tissue in the cecal wall is especially significant with regard to the B vitamins because the host is dependent upon bacterial synthesis for certain fractions of this group and for vitamin K Najjar and Barrett[733] point out that synthesis of the B group is brought about largely by microorganisms in contrast with vitamin C which is synthesized within the tissues Synthesis of B vitamins by the intestinal flora has been established by various investigators who have used several methods For example cecectomy diminishes the protective power of the feces against B deficiency except in instances where part of the remaining colon assumes the function of the cecum [4 3 733] Abdel Salaam and Leong (1938) found that mixed cultures from the rat caecum grown at 37 produced B vitamins They found that about 16 IU per g were synthesized by bacteria in young cultures and that the amount gradually diminished after 3 days incubation Low concentrations of antibiotics were considered by Eisenstark and Dragsdorf [77] to favor or actually stimulate the growth of bacteria because cecal filtrates from antibiotic fed birds stimulated bacterial proliferation more than cecal filtrates from controls It has been found that a strain of yeast cultivated in continuous transfers in a medium free of B vitamins synthesized vitamins B_1 and B [377] Quigley[8 0] collected data which show that biotin riboflavin thiamine nicotinic acid B_1 and other vitamins are synthesized by bacteria in the cecum of herbivores Other investigators have found that some of these B vitamins are synthesized in the cecum of rabbits [40 4 571 733]

Bacteria in the rumen and intestine are important in protein synthesis Bacteria in the rumen of sheep can use urea as the main source of nitrogen for the synthesis of 10 amino acids [19] Similarly protein may be synthesized

which copraphagy was practiced when the diet was hay and roots However, these caged rabbits regained their normal growth when fresh grass was included [19] Thacker and Brandt (1955) found no significant difference in the digestion of cellulose by rabbits permitted freedom of coprophagy, and those stanchioned to prevent ingestion of their feces [4 3] It has been reported that by coprophagy "rabbits convert their feed into meat as efficiently as swine" and that the gastric contents of rabbits has a pH of 2 0 to 3 2[45]" Cuthbertson and Phillipson"[19] point out that the psysiology of coprophagy is obscure because there is no distinct separation of food, as indicated by barium feedings

The importance of coprophagy is greater than is usually suspected Kulwich and co workers[571] calculated that rabbits, permitted to ingest their feces *ad libitum*, obtain about 83 per cent more niacin, 165 per cent more panthothenic acid, 100 per cent more riboflavin and more B_1 than when these animals are deprived of this practice Vitamin B deficient rats lived longer, and the young rats often made appreciable growth when they were permitted to reingest 70 per cent or more of their feces [877] It has been found that the prevention of coprophagy inhibits growth in young rats [40, 776, 877]

The effect of the administration of B_1 *per os* indicates that cobalt is an essential element for ruminants but that it is not as necessary for other herbivorous animals Horses, rabbits and herbivorous marsupials can do well on fodder seriously deficient in cobalt, whereas, sheep and cattle become debilitated [64] Attempts to produce cobalt deficiency in small laboratory animals and rabbits have not been successful [645] Certain failures to produce experimental cobalt deficiency in rabbits were attributed to habitual coprophagy [645] Coprophagy appears to be equally significant in the failure to produce cobalt deficiency experimentally in rats [645, 733]

Cecal and Appendical Flora

Some products of cellulose hydrolysis by intestinal bacterial are indirectly important in protein metabolism by furnishing media for the proliferation of bacteria Hawk and associates[41] state that there is disagreement on the exact extent to which cellulose is utilized in the animal' Twenty five per cent of ingested cellulose is considered to be utilizable by herbivora in contrast with less than 5 per cent by dogs and an insignificant amount by man [41]

The importance *of bacteria and Protozoa in the breakdown of* cellulose and pentosans in the rumen of cattle and sheep has been considered in many studies However comparatively few studies have been specifically designed to demonstrate the importance of the cecum of rabbits [423] Many

tease increase only 25 per cent or less.[779] Phage lysis of *E. coli* activates RNAase to degrade the endogenous RNA to dialyzable fragments.[637]

Bacterial activity is correlated with the presence of lymphoid nodules in the intestine. Peyer's patches are more numerous in the ileum than in the jejunum and duodenum in man and most mammals. The distal (storage) colon has solitary follicles but no aggregates. Perhaps this is a reason that most of the enormous number of bacteria, which compose about one third of the solid material of feces in man, have died in the unfavorable environment encountered in the distal colon.[890]

Histone Synthesis in the Intestine

Large lymphocytes in the germinal follicles in intestinal lymphoid tissue convert amino acids into histones prior to and during the process of mitosis. Dietary amino acids are usually considered to be carried by the blood to all tissues.[419] However, the amino acids which pass through the lymphoepithelium and some which pass through the mucosa of the intestinal villi are directly available for use in the synthesis of histones by large lymphocytes resident in the germinal centers of intestinal lymphoid tissues. Synthesis of nucleoproteins in large lymphocytes and in the cytoplasm of forming plasmacytes in the core of villi incorporates significant quantities of amino acids directly from the lumen of the intestine without their having passed into the blood stream.

The direct retention of amino acids becomes apparent when one considers that lymphoid tissue lymphocytes and plasmacytes in the core of intestinal villi are competing with the portal capillaries for the amino acids which pass through the intestinal epithelium. Protein synthesis in large lymphocytes occurs primarily in the germinal centers of the lymphoid structures and in the core of villi and represents an incorporation of amino acids into histones which are linked with the DNA in the nucleus.

Many small lymphocytes leave the intestinal lymphoid tissue by veins and lymphatics. Those entering lymphatics may be stored for a longer period in the pancreas Aselli or other mesenteric lymph nodes where some may transform into plasmacytes. Thus those entering the portal venous system may pass into the systemic circulation a little sooner than those taking the lymphatic route. Small lymphocytes are normally present in all circulatory fluids and large numbers disintegrate in blood, lymph and tissue fluid. Most cells produced by the mitosis of large lymphocytes are small lymphocytes which do not divide and consequently do not utilize these amino acids for their own proliferation.

The presence of histones in chromosomes from nuclei of lymphocytes has been determined by the isolation of nuclei (which involved prolonged exposure) in a Waring mixer or a colloid mill. This procedure yielded chroma

from urea in the rumen of cattle[2] It has been shown that proteins of bacteria and Protozoa from the rumen of cattle have biological values of 80 to 81 and that these proteins have percentage values of true digestability of 74 (bacteria) to 91 (Protozoa)[6 4] Nucleic acids in bacteria represent about 15 per cent of the dry weight[2 5] Voit[1062] believes that the nuclear material from 12 species of bacteria was thymonucleic acid (DNA) Schaffer and co workers (1922) extracted a substance from dehydrated bacteria which was considered to be a nucleic acid[50] Vendereley (1946) states that in one strain of *Bacterium aertrycke* 10 64 per cent of the dry weight was nucleic acid of which 7 0 per cent was RNA and 3 64 per cent DNA[795]

The amount of RNA, however, varies with the growth phase, and the ratios of RNA to DNA vary in different strains and species[11 1] Synthesis of DNA and RNA in bacteria is variously altered by factors such as the T phage, exposure to mustard gas[4 6] or x rays[8-] RNA in bacteria is a source of this nucleic acid for the synthesis of bacterial proteins within bacteria[113] The nucleotide content of bacteria in aged cultures is low, and increases when the bacteria are transferred to new cultures[8]

The significance of ruminal and cecal bacteria as a source of nitrogen is controversial Cuthbertson and Phillipson[19] review the literature and conclude that there is no doubt that some ruminal bacteria disintegrate and release amino acids but that some organisms do not disintegrate Digestion of ruminal Protozoa occurs in the abomasum,[19] or fourth stomach of ruminants

Bacterial proliferation in the cecum of rabbits may be related to the high DNA and histone content of the appendix in at least four ways (1) By aiding in the formation of B vitamins, some of which function as components of DNA, (2) by hydrolyzing cellulose to glucuronic acid or to one, two or three carbon compounds that can be utilized in the synthesis of glucose *d ribose of d deoxyribose (3) by disintegrating and supplying* DNA and RNA or their components, or (4) by furnishing enzymes or co enzymes for the synthesis of nucleic acids The cecum and appendix of rabbits could have several functions in increasing the amount of available protein from bacteria because goblet cells, which produce mucin are most abundant in these structures Mucus in the large intestine is believed to destroy bacteria in this part of the intestine in man[6 9 8 0] The contact of the great numbers of bacteria in the flask shaped mucous glands in the rabbit s appendix over a comparatively longer period of time than would occur in the intestines of most animals might be very important This retention of bacteria in the cecum and appendix could also facilitate the destruction of bacteria by phages For example infection of *Escherichia coli* with T2r+, T2r and T3 increases the activity of deoxyribonuclease (DNAase) from 40 to 100 per cent, but ribonuclease (RNAase) and pro

tease increase only 25 per cent or less[779] Phage lysis of *E coli* activates RNAase to degrade the endogenous RNA to dialyzable fragments[637]

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tin in the form of a "tightly packed sediment" by differential centrifugation in a molar solution of sodium chloride The tightly packed sediment was composed of coiled threads (residual chromosomes of Mirsky and Ris) [01] which represented 8 to 10 per cent of the mass and 4 per cent of the total nucleic acid content (chiefly RNA) of the original chromosomes, and were made up of "thicker, tightly coiled heterochromatic sections and more loosely coiled euchromatic regions' The opalescent and highly viscous supernatant fluid "was found to be rich in nucleohistone, which constituted 90 to 92 per cent of the mass of the chromosomes" When this supernatant fluid was diluted with 6 volumes of water, a fibrous precipitate "containing 15 per cent DNA and 55 per cent histone" was formed Most of the essential amino acids are present in the histones in nuclei of lymphocytes Thereby, the synthesis of histones in intestinal lymphoid tissues stores amino acids in an inactive form in small lymphocytes for later use by other cells

Histone synthesis in the cecum and vermiform appendix of rabbits probably contributes to the ability of rabbits to live and reproduce on a low protein diet The rabbit appendix is actually much larger than the human appendix When compared with total body sizes, the rabbit appendix is approximately 60 times as large as the human appendix The appendix of rabbits is composed chiefly of small lymphocytes Lymphoid tissues in the intestine are immediate sources of stored DNA as well as histone

Most tissues other than the lymphoid tissues store protein primarily for their own use Pfluger (1903) considered that the liver stored protein as well as glycogen and fat [603] Berg (1914) described 'protein storage granules" in the cytoplasm of hepatic cells (the so called 'protein storage granules of Berg) which Kosterlitz and Campbell (1947) believe are RNA [603] Protein storage by the liver was further stressed by Luck (1936) who observed an increase of 50 to 60 per cent in globulin, euglobulin pseudoglobulin and albumin in the liver of rats maintained on a high protein diet [603]

Disintegration of small lymphocytes in the intestinal lymphoid tissues and mucosa spleen and other portal organs provides histones which can be hydrolyzed to furnish most of the amino acids for the use of hepatic cells Human fibrinogen does not contain any amino acid which is not present in thymus histone but thymus histone contains lysine isoleucine and valine which are not usually reported to be present in fibrinogen [407] Fibrin however, contains lysine and phenylalanine in addition to all of the amino acids in fibrinogen [40]

Phenylalanine an essential amino acid, does not occur in thymus histone according to Harrow [407] However the presence of tyrosine partly compensates for its absence The immediate source of tyrosine for endocrine secretion probably would be provided from the plasma proteins in blood lymph

and tissue fluids Epinephrine is synthesized from tyrosine by the steps of decarboxylation which produce tyramine, followed by the oxidation of tyramine to produce arteranol and the methylation of arteranol [1084] Iodination of tyrosine results in the formation of 3,5 diiodotyrosine [1084] This is followed by the oxidative condensation of molecules and, thereby, the formation of thyroxin [1084] In addition tyrosine comprises 12 7 per cent of the total amino acids in insulin and 10 4 per cent in pepsin [407] Thymus histone should not be considered a direct source of tyrosine but its presence in thymus histone is important as an inactive reserve and as an indirect source Disintegration of lymphocytes is also a potential source of tyrosine for plasma proteins such as fibrinogen, which contains 5 8 per cent tyrosine gamma globulin, 6 8 per cent or serum albumin 5 3 per cent

Lysine metabolism differs from that of other amino acids Lysine (11 7 per cent) and arginine (12 9 per cent) are the most abundant amino acids in thymus histone with leucine (7 4 per cent) next [41] The concentration and storage of lysine in thymus histone is significant because lysine is the only amino acid which is not involved in reversible nitrogen shifts Lysine is the only indispensable amino acid which can not be replaced since it is unable to obtain nitrogen from any other amino acid [60, 1084] The percentage of lysine in plasma proteins varies Human serum albumin contains almost as high a percentage (10 4) of lysine as occurs in thymus histone (11 7) However human gamma globulin contains only 6 7 per cent and human *fibrinogen does not contain lysine Human fibrin however contains* 8 8 per cent lysine[412] and 6 0 per cent phenylalanine which are two amino acids present in fibrin but absent in fibrinogen [41]

Arginine in histones within the nuclei of intact small lymphocytes is stored and protected against the action of hepatic arginase[1084] into urea Thymus histone contains almost twice the percentage of arginine present in several plasma proteins or in beef muscle Human fibrinogen contains 7 9 per cent human serum albumin 6 0 human gamma globulin 4 8 and beef muscle only 7 2 compared with the 12 9 per cent in thymus histone [407] The high percentage of arginine in thymus nucleohistone suggests a possibility that histones in lymphocytes may serve as a storage and transportable source of arginine for histones or protamines in other cells as well as a source for synthesis of creatine

The absence of some amino acids in thymus histone may be just as significant in the metabolism of lymphocytes as is the presence of other amino acids Cysteine glutamic acid and glycine are usually not mentioned as being present in thymus histone [41] However the formation of pyruvic acid from several of the amino acids and the synthesis of amino acids from pyruvic acid provide a cycle by which histone in the nuclei of small lymphocytes can be a source of non essential amino acids The amino acids in thy

mus histone which may be deaminated to form pyruvic acid, include some possibly leucine, valine and threonine[412, 1084] Pyruvic acid from these amino acids can be incorporated in the synthesis of other amino acids, or they may be deaminated to form glucose[1084] The incorporation of the amino acids in histones, therefore, represents a potential source of glucose and glycogen as well as a source of pyruvic acid for numerous and varied reversible reactions, such as those in lactic acid reactions in the citric acid cycle, in the processes of the formation of acetic acid and active acetate,[1041] *and even in the metabolism of adenosine triphosphate (ATP) through* oxidations by the tricarboxylic cycle[1084]

APPENDIX OF MAN AND RABBIT

The occurrence of a true vermiform appendix (appendix caeci, appendix vermicularis caeci) is fairly delimited in phylogenetic distribution Unfortunately, the use of the term is not well delimited Some investigators fail *to differentiate between the cecum and its vermiform process in mammals* Some writers even use the term "appendix," or "cecal appendage," synonomously with the term "cecum" One author reports that the "appendix" of the dog was studied[505] Stohr depicts typical early (2½ and 5 days after birth) stages of appendical nodules in the rabbit which are captioned "sections through the wall of the caecum"[37] Oppel's[759] and Wiederscheim's[109] statements that some of the rodents have a vermiform appendix were made before taxonomists had agreed that rabbits and their relatives are no longer duplicident rodents but belong to an independent order, Lagomorpha It is not so easy to explain why investigators have referred to lymphoid tissue in the appendix of the guinea pig[91] and stated that "appendicitis without the infection" can be produced in dogs[901]

In all probability, no representative of the orders Rodentia or Carnivora has a vermiform cecal appendix whereas at least in North America, all members of the order Lagomorpha (rabbits hares and pikas) are characterized by the presence of a true, well developed lymphoid, vermiform appendix Other forms including man which have a vermiform appendix apparently all belong to the Anthropoidea[730, 759]

The appendix *of both man and rabbit secretes a considerable amount* of fluid The catheterized normal human appendix secretes at least 1 to 2 ml of fluid daily[1071] As much as 20 ml of fluid may be collected from the rabbit s appendix in 6 hours The fluid from the rabbit s appendix was distinctly alkaline (pH 7 2 to 8 68) and contained erepsin sucrase and maltase[1071] The appendical fluid in man is thick and yellowish and contains degenerated cells, mucin and bacteria[8 0] Antibiotics injected into the lumen of the rabbit s appendix did not affect the rate of secretion or the

"quality of fluid secreted."[1071] It is interesting to note that a low incidence of appendicitis is considered to occur among uncivilized natives who subsist on a high cellulose diet and that the incidence increases when these people adopt a diet common to civilized people.[901]

Appendix of Man

The vermiform appendix of man differs somewhat in structure and function from that of the rabbit. The human appendix is about 3.5 in long,[450] but this varies.[335] It has a small lumen which commonly becomes occluded with detritus by middle age.[335, 1071] The human appendix has a thickened mucosa, a dense, fibrous submucosa and a more or less confluent rather dense array of solitary lymph nodules.[493] We[0] have observed, and Maximow and Bloom[690] have reported, that these nodules resemble those in the human tonsil rather than those of other intestinal lymphoid structures and that there is a rich supply of crypts of Lieberkuhn which are partly embedded in the internodular lymphoid tissue.

An individual appendical nodule in the adult human appendix is spheroidal in form.[493] Some of the nodules are surmounted on the luminal side by a small area of luminal epithelium modified to form a lymphoepithelium. In the adult rabbit's appendix the individual nodule is an elongated, conoid structure 3 to 4 mm in height.[20] The lymphoepithelial apex of the nodule forms the bottom of a flask-shaped mucous gland, which is confluent with the lumen of the appendix by a small orifice.

The appendix and cecum of the human fetus maintain an equal circumference until the 5th month *in utero* when the appendix ceases to grow. However, the cecum keeps pace with the colon. At the cessation of growth the wall of the appendix "becomes infiltrated with large numbers of lymphocytes."[495] The lymphoid tissue is poorly developed in the appendix of newborn infants.[26] Nagoya (1913) noted that in the human infant the lymph nodules in the appendix were almost completely developed by the 2nd month after birth.[1071]

The human appendix has much less lymphoid tissue than that of the rabbit. The lymphoid tissue in the human appendix is characteristically localized in spheroidal nodules surrounded on all sides by a very dense, fibrous connective tissue. Any direct connection of these nodules with the lumen of the human appendix is by a lymphoepithelium similar to that in the crypts of the tonsil. Another seldom described difference between the human and lagomorph appendix is that little digested material (especially amino acids and nucleic acids) can pass directly from the lumen of the human appendix into the lymphoid nodules. A rather massive fibrocollagenous barrier surrounds most of the individual nodules in the human appendix

This barrier blocks direct absorption *from the appendical lumen The* architecture of the human appendix does not justify classing it with the intestinal lymphoid tissues

The human appendix does not have flask shaped mucous glands which are characteristic of the rabbit appendix If the function of the rabbit appendix is related to the activities that occur in the long cecum, it is easy to visualize that the human appendix is a vestigial organ because the cecum of man has a very limited capacity and, consequently, does not adequately provide for storage and bacterial decomposition of cellulose or for adequate absorption of the products of intestinal biosynthesis Oppel[759] shows that all forms belonging to the suborder Anthropoidea from the gibbons to man have a cecal appendix He states that Flower (1872) gives the approximate length of the vermiform appendix of gibbons (hylobates) as about '3 Zoll (9 0 cm), of the chimpanzee, 15 0 cm, and of the gorilla, 25 5 cm However, Muthmann[730] states that the cecum terminates in a conoidal tip in the Rhesus monkey

Probably due to its dense fibrocollagenous submucosa and the more scattered arrangement of its lymph nodules, excised human appendices were not ruptured by internal pressures up to 2400 cm of water, but rabbit appendices ruptured when the pressure reached 120 to 150 cm of water[1071]

The structural and physiological relations of the normal human appendix are obscured by the fact that some investigators believe that it is a vestigial organ which serves no useful purpose[1] A comparative study of natives of French equatorial Africa was interpreted to indicate that the appendix is in the process of evolution rather than being a vestigial organ[774] The lumen commonly becomes occluded or obliterated, and the structure atrophies before the age of 50 in about half of the people,[335, 1071] or is extirpated before middle age is reached Thus, until recently, the very old statement that the functions of the human appendix vermiformis are unknown [5] has sufficed Indeed the clinical implications inspired a surgeon to caution that the presence of the appendix in a man is a sword of Damocles hanging over his head[10, 1] Since the appendix of the fetal[730, 741] and newborn infant[335] arises from the apex of an accuminately conoid cecum it may *be surmised that the appendix of early man was, essentially, a functional organ as it is in the lagomorphs However, the appendicocecal union* in the orangutan has far more similarity to that of the adult than to that of the newborn of man[730, 741]

The presence at the orifice of the appendix of one or more types of mucosal folds (Gerlach s valves or Nanniga s folds) in about 80 per cent (81 5 per cent of 526) of the people has been known since 1847[1071] but the significance of these valve like folds remains little understood However Wangensteen[1071] suggests that the function of these mucosal folds in man

is probably to prevent intestinal contents from entering the appendical lumen

Appendix of Rabbit

The appendix of the rabbit is of generous size, and remains patent and functional throughout the life of the individual It is composed chiefly of a dense, specialized intestinal type of lymphoid tissue [0] The appendix reacts to various systemic stimuli such as inanition or starvation[9 1 471] and the administration of arsenicals [59] or benzene,[514] as do the other lymphoid tissues In addition to its significant lymphocytopoietic function it is an important structure for the promotion of biosynthetic activities of the intestinal flora and for the absorption of the products of intestinal biosynthes s

Intestinal lymphoid specialization is greater in the appendix of lagomorphs than in any structure in other common laboratory mammals Most of the work on the histology of the rabbit appendix has been primarily concerned with the structure of the lymphoid tissue [37 0 498 585 1086] The lymphoid nodule and its associated flask shaped mucous gland form an interdependent structural and functional unit [0] in rabbits (snowshoe white and black tailed jacks, several domestic and a species of western cotton tail) The cecum of 159 preserved adult rabbits, chiefly New Zealand white, averaged 16 5 in [994] and the length of the appendix ranged from 4 to 5 in [35] The wall of the rabbit appendix is very thick due to the increased thickness of the mucosa and the very large and long nodules The wall is composed chiefly of conical lymphoid nodules each surmounted by the characteristic flask shaped mucous gland which lies within the thick mucosa [702] The tunica muscularis of the rabbit s appendix is as thin, or thinner than it is in the cecum but it maintains peristaltic movement in anesthetized rabbits [51]

The relative size of the New Zealand white rabbit s appendix in terms of body weight may be expressed by the term 60 times as large as the human appendix The appendix of the rabbit was 1 to 1 5 in longer and its maximal diameter was nearly 4 times that of the appendix of a normal 20 year old man The wall of the fixed rabbit appendix is as thick as the human appendix and contains much more lymphoid tissue whereas the lymphoid tissue of the human appendix is limited to single more or less independent to confluent nodules which are embedded in dense fibrocollagenous connective tissue

The lymphoid nodules in the appendix of lagomorphs as illustrated in domestic hares [0] cotton tail and snowshoe rabbits black and white tailed jack rabbits and pikas are a specialized type of intestinal lymphoid tissue The lymphoepithelium is in direct contact with the lumen of the appendix

This barrier blocks direct absorption from the appendical lumen. The architecture of the human appendix does not justify classing it with the intestinal lymphoid tissues.

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as do other lymphoid tissues Thus, starvation or severe inanition depletes all intestinal and other lymphoid tissues about equally [9, 251, 490] However the thymus is the most sensitive of all lymphoid tissues Arsenicals,[259] ben zene [514] total body irradiations, [86] urethane, nitrogen mustards and corti sone [06] deplete lymphoid tissue throughout the intestinal mucosa These agents did not have a selective action on any one form of lymphoid struc ture such as solitary follicles or peripheral nodes, or on lymphoid tissue in any one area such as that in the ileum Effects of such agents upon the lymphoid tissue are systemic rather than direct Therefore they have about the same effect on lymphoid tissue in the appendix as on other lymphoid tissues

Strong peristaltic waves pass along the appendix of rabbits under Dial (Ciba) anesthesia As a rule, we found very little residue of food in the lumen of the appendix and, relatively, much less or none in the flask shaped mucous glands [0, 514] These flask shaped glands appear to have no obvious provision for filling and emptying themselves, but the pumping action of peristalsis could easily fill and empty them by alternately distending and compressing them Observations on the living appendix of the rabbit sug gest that appendical peristalsis causes the flask shaped glands to operate by suction to change the flow of material The flow of fluid into the flask shaped glands increases the diameter of the lumen of these glands during the period between contractions of the appendix When the central lumen of the appendix is distended, the wall of lymphoid tissue becomes thinner As this process occurs, the ostia and lumen of the flask shaped glands are opened The central appendical lumen is smaller during peristaltic contrac tion, this decrease causes the wall to become thicker and the lumen of the flask shaped glands to constrict By this process material from the flask shaped gland is forced into the lumen of the appendix At the same time material in the terminal end of the central lumen may be moved toward the cecum Apparently there is no mechanism in the rabbit s appendix com parable to Gerlach's mucosal fold in the human appendix However the spiral valve of the cecum probably acts as a kinetic and valvular structure in controlling the entrance and egress of appendical contents At appendec tomy we have observed rabbit appendices that were virtually empty and others that were nearly filled with cecal contents

Wangensteen[1071] points out the possibility that pinworms may cause appendicitis in man Cecil and Bulkley believe that pinworms are responsi ble for an appreciable percentage of cases [6, 5] Ackerman[1] and Bell [9] state that pinworms occur as frequently in normal as in pathological appendices and that appendicitis is not caused by the presence of these worms in the human appendix We have never seen any indication of inflammation in appendices which contained pinworms in hundreds of embalmed rabbits dissected in the laboratory Particular attention was paid to a number of

because it forms the bottom of the flask shaped mucous glands These mucous glands are blindly ending extensions of the central lumen of the appendix Substances may pass from the lumen of the rabbit's appendix directly to the intestinal lymphoid tissue in the wall of the appendix by three routes (1) via lymphatics that arise as capillaries in the richly glandular interostial mucosa (between ostia of flask shaped mucous glands and crypts of Lieberkühn) and empty into a perinodal sinus, (2) via lymphatics that arise as capillaries in, or perforate (as intercellular canaliculi) the lymphoepithelium and empty into a large perinodal sinus, and (3) by merely passing through the lymphoepithelium *or out of the lymphatic capillaries into* the intercellular fluid within the nodule[20] This lymphatic specialization assures a constant, nearly inexhaustable supply of lymph which is directly available to the extracellular fluid in the massive lymphoid nodules in the rabbit's appendix

The mucosa of the appendix of the rabbit is well supplied by an elaborate commodious lymphatic drainage system The appendical lumen, flask shaped mucous glands, crypts of Lieberkühn, the intervening mucosa and the apical, or most active half, of the conoid nodules are drained by the *dense arborization of fine tributaries of the wide perinodular collecting si*nuses In turn, the sinuses empty into the large appendical lymphatic in the mesoappendix[20] This lymphatic extends along the appendix as the cecal lymphatic It receives tributaries from the cecum and eventually empties into the pancreas Aselli From these mesenteric nodes, the lymph passes into the cysterna chyli

The available literature on the development of lymph nodules in the rabbit's appendix shows wide variation Stohr depicts a well defined, conoid lymph nodule from a rabbit 2 5 days after birth and an adult type nodule *from the appendix of a 5 day old rabbit*[37] *Latta*[585] *found no indication of* nodule formation in new born or earlier stages' He found only free cells beginning to be massed in the location of future nodules' in 2 to 3 day old young

In fetal rabbits 19 days after mating the mother,[0] lymph nodules and flask-shaped mucous glands were definitely delimited in connection with abortive villi near the tip of the appendix These nodes and glands were well outlined as aggregates of *lymphocytes at the base of an epithelial* lined cavity which was destined to become the lumen of a flask shaped mu*cous* gland It was evident that the nodule and the flask shaped gland developed concurrently in the base of an abortive villus In 7 day old young these nodules resembled those of adults in general appearance Incidentally, the appendical villi had almost disappeared by the end of the 2nd week at which time the nodules were similar in form to those in an adult rabbit[20]

The rabbit appendix reacts to various systemic conditions and stimuli

to the normal level a few days after appendectomy Variations in the duration of lymphopenia were ascribed to differences in the ability of the remaining tissue to compensate for the loss of the appendix Age, breed or sex were not the causes for compensatory differences, because litter mates of the same sex differed as much in this regard as unrelated animals

Hypertrophy of lymphoid tissue following appendectomy affected only the sacculus rotundus, the tonsilla ileocecalis major and the Peyer s patches Appendectomy did not cause significant changes in the lymphoid tissue in the lymph nodes, pancreas Aselli, spleen or thymus The simple hypertrophy of the sacculus rotundus and tonsilla ileocecalis major resulted in an increase of one fourth to one third in rabbits that had been appendectomized 2 months previously The nodules of the tonsilla ileocecalis major varied considerably in size In some of the appendectomized rabbits, these lymph nodes were much enlarged, whereas in others they were smaller but their capsules were unusually very thick as compared with those of the enlarged nodes Thus, subsequent to appendectomy, hypertrophy rather than hyperplasia[364, 7 3] occurred Peyer's patches and the tonsilla ileocecalis minor were the least affected of the hypertrophied lymphoid tissues in the appendectomized animals The number of Peyer s patches in the ileum did not change during the period of 2 weeks to 2 months after the operation The number of patches showed no relationship to sex breed or experimental conditions as compared with the number in 3 controls and the number obtained from examining the intestines of 14 male and 6 female embalmed animals The hyperplasia of lymphoid tissue in the sacculus rotundus and the first (from the ileocecal valve) Peyer s patch represents only a very slight replacement of the lymphocytopenic and storage functions of the appendix

Nutritional deficiencies [83] and hunger must also be carefully considered in evaluating these observations Under adverse conditions the appendix of the rabbit decrease more rapidly in weight and size than the lymph nodes or other tissues of the body [490] except the thymus Inanition also decreased the number and mitotic activity of lymphocytes in the remaining lymphoid tissue in the dog [781] The hypertrophy of the sacculus rotundus and Peyer s patches in appendectomized rabbits indicates an increase in availability and concentration of nucleoproteins and/or their components Hypertrophy of the sacculus rotundus tonsilla ileocecalis major and minor after appendectomy suggests that one function of this lymphoid tissue is to assimilate substances which become concentrated in the distal ileum while the ileocecal valve is temporarily closed as fecal material is being passed from the cecum into the colon The slight hypertrophy of the first Peyer s patch from the ileocecal valve indicates that there may be regurgitation from the cecum into the proximal end of the ileum

experimental animals We did not see either macroscopic or microscopic indications of appendicitis or even inflammation in 22 New Zealand rabbits which had numerous pinworms in the appendix

APPENDECTOMY IN RABBITS

The appendix of the rabbit has advantages over other intestinal lymphoid structures for studies on the function of intestinal lymphoid tissue[514] and, also over most lymph nodes and the spleen[230, 514] In the first place, appendectomy in the rabbit does not significantly disturb blood volume, because it is a terminal structure, nor does it cause any degree of lymph stasis which usually follows the removal of lymph nodes Furthermore, removal of the appendix of rabbits removes about one third, by weight, of all the constant lymphoid structures, including the spleen, thymus and 22 paired lymph nodes[05, 514]

Appendectomies were performed on rabbits in order to evaluate the function of the vermiform appendix as a lymphoid organ[514] Peripheral lymphopenia *was the only significant effect* observed in the appendectomized rabbits This result indicates that one function of the rabbit appendix is to produce small lymphocytes and to release these cells into the blood stream Removal of the appendix did not interfere with assimilation and growth as indicated by the insignificant changes in the weights of adult rabbits following appendectomy The lymphopenia occurred in every rabbit after appendectomy, and was detected within 4 hours after removal of the organ and often persisted in diminishing severity up to 3 weeks Anesthesia produced no significant change in the number of blood lymphocytes during this period as compared with the count of the previous day However appendectomy decreased the lymphocytes in the blood stream within 4 hours to less than 50 per cent of the normal number The suddenness of the lymphopenia indicates a rapid turnover of lymphocytes and that the appendix is a reservoir for circulating lymphocytes This decrease in blood lymphocytes following lymphoid ablation agrees with the findings of other investigators[8, 6, 893] particularly with the decrease in lymphocytes in the rabbit following extensive lymphoid ablations such as removal of the entire intestine[88] or of the sacculus and pancreas Aselli[893]

Appendectomy did not consistently change the total leukocyte or erythrocyte value In 1 rabbit, the postoperative total white counts were the same as the preoperative counts in 3, the postoperative counts were significantly higher and in the remaining 4 considerably lower The number of erythrocytes, likewise did not reflect any significant trend after the operation

The number of lymphocytes following appendectomy remained at a subnormal level *for the individual for a period of about 4 weeks* in 3 rabbits In 3 other rabbits the lymphopenia was temporary and suddenly returned

Lymphocytes become "most abundant after inflammation has lasted several days" and may remain for some time after the lesion apparently has healed [633] Some of these lymphocytes transform into plasmacytes [633] The lymphocytes and plasmacytes have the function of storing and supplying nucleic acids, adenosine triphosphate (ATP), histones, certain enzymes and other proteins and certain minerals for use by the proliferating cells in the healing or last stage, of the subchronic or chronic inflammatory process Eosinophils may be present in the blood, and both eosinophils and mast cells usually are present in the tissues of the inflamed or adjacent area

Mast cells may function directly and indirectly in wound healing The granules of mast cells may release histamine and serotonin[334] to produce hyperemia and increase capillary permeability—a process which increases proteins enzymes, and other substances in the area of wound healing Cramer [13] records frequently seeing 'accumulations of mast cells in the neighborhood of areas of massive epithelial hyperplasia' The significant relation of mast cells to hyperplasia and wound healing would probably be by increasing capillary permeability rather than by being a source of collagen It has been pointed out that chemical and histochemical observations concentrate on a mucopolysaccharide which appears to be important in bone healing' and that 'mast cells may be the means by which the acid mucopolysaccharide is concentrated [453] However, the number and distribution of mast cells in granulation cysts produced by turpentine indicate that mast cells are not numerous enough to be an important source of acid mucopolysaccharides (AMP) Their distribution does indicate that they might be indirectly significant for collagen formation by increasing capillary permeability

The polymorphonuclear leukocytes are the first of the infiltrating cells to arrive at a site of inflammation [633] Neutrophils are especially attracted by acute infectious processes and respond in great numbers to the presence of pus cocci [8, 3, 746] certain other microorganisms and certain chemicals [633] The macrophages (histiocytes) contain trypsin and proteases[603] which enable these cells to digest engulfed proteins However phagocytes die and free the components of the engulfed material which are then reavailable for use by other cells Thus phagocytes contribute toward the repair process which follows the acute phase of tissue injury [77]

In 1888 Leber introduced the concept of positive leukocytic chemotaxis in response to infection by microorganisms specifically by the fungus Aspergillus [663] Later it was shown that leukocytes are positively chemotactic to sterile products of injured tissue' as well as to toxins of microorganisms [663] Metchnikoff explained the time sequence in the infiltration by leukocytes lymphocytes and presumably plasmacytes into an area

12

Inflammation and Wound Healing

Several cells in inflammations include lymphocytes, plasmacytes, mast cells, neutrophils, eosinophils and monocytes The cause and nature of the inflammatory lesion alter the kinds and numbers of these various cells In turn, the order of infiltration and the order of disintegration of these cells are the means by which one biochemical process predominates over another and by which a series of changes in a delimited area proceeds in an orderly manner

The number and kinds of these cells fluctuate in accordance with variations in the nature of the inflammation, and the rates of regeneration and wound healing Inflammations are usually designated as acute, subchronic (subacute) or chronic depending upon the cause and duration of the condition Acute inflammation lasts for a few days or two or three weeks," and is usually characterized by heavy infiltrations of the polymorphonuclear leukocytes Chronic inflammation lasts for months or years[663] and is characterized by infiltrations that are predominantly composed of lymphocytes and monocytes Subchronic, or subacute, inflammation[239] are terms used to indicate an inflammatory condition of shorter duration than chronic inflammation but more severe than the acute stage[239, 663]

Inflammations serve the purposes of destroying eliminating or limiting the spread or effects of the injurious agent and of repairing injury or replacing the necrotic or damaged tissues [71, 6, 633, 663] The onset of inflammation is preceded and attended by hyperemia and increased capillary permeability The sequence of the four cardinal signs of inflammation (which describe the changes in the involved area) is rubor (redness) calor (heat) tumor (swelling) and dolor (pain) [6, 0, 633, 663] Redness and heat result from hyperemia and increased capillary permeability with intercellular exudation and edema which are chiefly responsible for the swelling and pain

The order of the infiltration of blood cells is usually the same regardless of the causes of the inflammation Polymorphonuclears are the first cells to emigrate into the inflamed area and monocytes arrive next

inflammation and in various injuries is generally accepted [396, 458, 849, 867] However, Angevine[14] cautions that histamine is "only one factor and that its action must be considered in relation to the other alterations that may occur" Zweifach[1139] warns that the histamine is not an all inclusive answer to changes in capillaries Menkin[68, 683] reports the isolation of certain fractions from aseptically produced exudate Each fraction has a special part in inflammatory processes, thus leukotaxine increases capillary permeability, and a leukocytosis promoting factor (LPF) causes the emigration of leukocytes [683] Hamilton[396] believes that the axon reflex' upon the arterioles controls the vasodilating effect There appears to be little that is definitely known about the mode of action of hormones in controlling inflammatory processes However, it is generally accepted that the 'reactivity potential of the tissue to an irritant may be conditioned by hormones [10, 4]

Halpern and co workers[330] concluded that histamine occurs in living organisms 'combined (HC) within the cells 'labile' (HL) and 'free (HF) They believe that the inactive, combined and labile forms of histamine are activated by conversion to the free forms of histamine, such as that which occurs normally in blood by liberating substances Code[174] found that in centrifuged normal and in passively sensitized rabbit blood the white cell layer, the buffy coat, has repeatedly 'been shown to be the source of released histamine' and that 70 to 100 per cent of the histamine in the blood of man, horses cattle goats and rabbits was in the white cell layer of centrifuged samples of blood By the process of eliminating the lymphocytes monocytes and platelets as containing very little or no histamine, he concluded that the myelocytes of the granulocytic series contain the most significant amount of the histamine found in the white cell layer of human and other centrifuged blood In further support of this tenet he points out that the blood of patients with myelogenous leukemia had the highest histamine content of any blood samples examined In this connection it is interesting to note that Dale (1936) found that the platelets of rabbits contain a high amount of histamine [86] which is at variance with the finding of Code [174] However the two investigators agree that blood platelets of the horse do not contain histamine

Neoplastic Growth

One prerequisite for localized reparative or even neoplastic, growth is a sustained increase in the supply of proteins and certain enzymes The most common method of increasing proteins in tissue fluid is by hyperemia and increased capillary permeability There are a number of endogenous substances and conditions which are capable of causing increased capillary permeability or spreading effects These include histamine hyaluron

of inflammation in terms of a sort of differential chemotaxis existing in the extravascular fluids[6, 5] Thus, early injury of tissue cells released substances positively chemotactic for polymorphonuclear leukocytes The early action of this positively chemotactic substance was to provoke a stickiness at the surface of endothelial cells which inhibited passage of the leukocytes through the blood vessel Thus, the sludged blood facilitated the emigration of these cells from the blood into the extravascular fluid With the passage of time, the changing extracellular substance became less positively chemotactic for leukocytes but increasingly positive for lymphocytes[6] The response of neutrophils and eosinophils to sterile necrotic tissue may be most important in view of the finding that horse radish oxidase, in the presence of hydrogen peroxide, can oxidize a variety of proteins which contain tyrosyl groups[944]

HISTAMINIC HYPEREMIA

Increased capillary permeability is one of the earliest manifestations in inflammation Histamine, as well as histamine like substances, increase capillary permeability and increase the protein content of edema fluid[390] Apparently, Marshall[64] does not consider histamine to be an activating agent, but he *does recognize that edema is dependent upon capillary dila*tion and that increased capillary permeability is a trigger mechanism which sets off the transudation of edema He attributes the formation of edema to the result of microcirculatory disease which causes considerable dilation of the terminal vessels

Histamine may be a potent factor in determining the relative number of plasmacytes to lymphocytes in subacute and chronic inflammatory lesions because dilation of blood vessels increases the permeability of capillaries so that protein rich plasma readily leaks through the capillary walls into the surrounding tissue[390, 1048] However histamine affects different species of animals in different ways Dale and Laidlow (1911) point out that the injection of histamine 'causes the typical symptoms and pathology and anaphylactic shock,' but that the actual stasis is variously obtained in different species of mammals Thus the pulmonary arteries of rabbits are contracted the hepatic veins of the dog are constricted and the bronchia of the guinea pig are contracted[1048]

Histamine occurs in all normal mammalian cells[390, 1048] Therefore tissue destruction or injury releases histamine[458] Lewis (1927) found that the injection of histamine produced reactions identical with "the so called triple response which is produced by injurious action of physical thermal or chemical agents[1048] on human skin His results were later verified by other investigators[390, 867]

The ability of released histamine to increase capillary permeability in

well as caused by, gastric or enteric disturbances Even a shaved and varnished skin area on an experimental animal may have damaging effects on the wall of the stomach and suppress the formation of hydrochloric acid Achlorhydria is associated with increased free histamine and marked dermatoses[1047] Thus it appears that gastroenteritis may cause increased free histamine that increased histamine may cause gastroenteritis, and that either of these conditions prevailing in the stomach or intestines may cause various dermato es and, conceivably lesions or alterations in other structures Similarly, it would appear plausible to consider many of the cases of light hypersensitivity, especially those cases characterized by acute dermatosis as being due to the so called telergic activity of hi tamine However porphyrin appears to be the suspected substance al though time has increased the controversy over whether or not porphyrin is at all related to light sensitivity[1048]

Rouget Cells

Rouget cells have been considered to function in contraction of capil laries but their significance in normal or pathological tis ues has not been established Rouget cells are variously known as adventitial glomus pericapillary and perithelial cells pericytes and true pericytes Some his tologists state that these cells are merely among the pericapillary ele ments[43]

The occurrence and distribution of Rouget cells are not very well delimited as to species organ or structure Zweifach (1940) states that Rouget cells are never associated with capillaries, but when present they are invariably situated on precapillary arterioles[43] However these cells are known to occur "on a large number of capillaries attached to the endothelial wall,"[56] especially in amphibians Rouget cells have been studied in the tail of living larvae of the newt and frog in the exci ed living tongue and nictitating membrane and in the web of the foot of frogs[70, 565] Rouget cells occur in all tissues of the human eye the mesentery of man and rabbit[56] the amnion of heep embryos and the muscular coat of the small intestine of the mou e[70] Also mast cells are present on arteries and veins of the kidney and in the tis ues of fishes, reptiles, birds[70, 56] cats, rabbits and men on the capillaries of guinea pigs, on true arteries (vasa efferentia of renal bodies) in dogs guinea pigs men and rabbits[70, 56] and on blood vessels in the worm Nereis[780]

The work of several investigators especially Krogh and as ociates indi cates that Rouget cells effectively constrict capillaries[70, 65] Rouget cells have fibrillae which are quite similar in form and staining reaction to the myofibrillae in the smooth mu cle cells of arterioles For this and other reasons Rouget cells are often considered primitive smooth muscle

idase, leukotaxine, ATP, and other substances in exudate,[68] Casey's Brown Pearce carcinoma extract,[983] Carminati s alcoholic extract of homologous tumors,[983] Boyland and McClean s tumor extract[983] and local anoxia [397] Possibly, any one or more of these agents may be involved, however, histamine is present in virtually every normal cell and may be activated or freed by a wide variety of agents Since it is extremely effective in increasing capillary permeability, we believe that histamine is probably one of the most important agents in increasing capillary permeability By this means histamine may divert proteins to areas of wound healing or chronic inflammation

We have not been able to find a reference to experimental or theoretical work indicating that histamine synergizes or contributes to spontaneous or induced neoplastic growth Heinlein (1936) used histamine on cats and rabbits for 'rather long treatment', and Hay and co workers (1943) applied a beeswax mineral oil preparation of histamine dihydrochloride daily to 2 calves, 1 monkey, 3 rabbits and 1 woodchuck for 20 to 59 days without producing a tumor [409] Ellinger (1939) reported that ultra violet irradiation enhanced the growth of the Ehrlich mouse carcinoma This increa ed growth was assumed to be the result of histamine, activated by the ultraviolet rays [983] Segal (1939) found the histamine content of blood samples from cancer patients within normal values but irradiation of the neoplastic growths resulted in increased blood histamine which was attributed to irradiation induced necrosis [983]

Telergic Relations of Histamine

The Arthus (1903) or Schwartzman (1928) reaction [867] the dye tests used by Rogouriez (1885),[564] or the trypan blue test as developed by Spagniol (1927) [867] Menkin[68] and others offer bases for an approach to the understanding *of certain supposedly telergic relations of histamine to* certain types of inflammatory lesions The relation of histamine to chronic inflammation is not always a localized reaction although many investi gators have shown that most injuries cause the release or activation of *histamine at the site of the injury*

It is now generally known that gastroenteritis or other injury to the gastric *or intestinal wall the presence of certain bacteria in the colon* an infected tooth or nearly any focal infection[1018] may result in food allergization with the production of various and often extensive derma toses [1047] A number of investigators attribute many of the persistent derma toses to gastritis, enteritis excessive intestinal putrefaction[315] and in testinal intoxication, any or all of which are in turn caused by the increased formation of histamine or imidazolemia Also, these reactions appear to be reversible, that is, skin lesions may be responsible for as

well as caused by, gastric or enteric disturbances Even a shaved and varnished skin area on an experimental animal may have damaging effects on the wall of the stomach and suppress the formation of hydrochloric acid Achlorhydria is associated with increased free histamine and marked dermatoses[1047] Thus, it appears that gastroenteritis may cause increased free histamine, that increased histamine may cause gastroenteritis, and that either of these conditions prevailing in the stomach or intestines may cause various dermatoses and, conceivably, lesions or alterations in other structures Similarly it would appear plausible to consider many of the cases of light hypersensitivity, especially those cases characterized by acute dermatosis, as being due to the so called telergic activity of histamine However porphyrin appears to be the suspected substance, although time has increased the controversy over whether or not porphyrin is at all related to light sensitivity[1048]

Pouget Cells

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cells[70,563] Results of extensive investigations seem to leave no doubt that Rouget cells function actively on certain capillaries in amphibians in vasoconstriction. However, several investigators question the ability of Rouget cells to "constitute the contractile elements proper" or to be at all essential for the contraction of capillaries or small blood vessels.[43,153,311,31,660,716,810,1135] Barnett[43] sums up the discussion by stating that "it seems to be the concensus *of more recent investigators that capillary* contractility is independent of the Rouget cells." Forbus[31] suggests that Rouget cells play an important part in capillary permeability. There is a correlation between the intensity and rapidity of capillary reaction, and the number of normal Rouget cells on the wall of small blood vessels and capillaries.

The slower reacting capillaries have small and very few Rouget cells, whereas capillaries which react rapidly and strongly were furnished with numerous Rouget cells.[63] Schaly (1926) found a linear correlation, because mast cells in the eye are distributed an average of 30 μ apart on capillaries in the choroid, *50 μ near the equator and 74 μ* behind the periphery of the retina.[364]

Factors Inhibiting Exudation

There are several means by which increased capillary permeability or fragility can be reduced or inhibited. The most obvious method is the inactivation or disappearance of the causative agent. However, there are several other means by which capillary permeability is decreased. Bioflavinoids (vitamin P, citrin and rutin, from oranges and lemons) reduce conditions of increased capillary permeability or fragility but their mode of action has not been established. The administration of the bioflavinoid, hesperidin methyl chalcone notably reduced hyperpermeability in animals in which excessively increased capillary permeability was experimentally induced. The addition of ascorbic acid markedly potentiated the effect of the hesperidin.[9] The chalcone form of hesperidin and a protein form an oxidation reduction couple.[39] Ascorbic acid is an active anticoagulant[39] and antiedemic.[9] It plays an essential part in the oxidation reduction system of tissue respiration.[9] Probably ascorbic acid functions as a coenzyme and "contributes to the development of antibodies and the neutralization of toxins in the building of natural immunity to infectious diseases."[9]

Significance of Edema Protein

Edema fluid is essentially a capillary filtrate. Normally, there is maintained a *homeostatic* state in accord with Bernard's concept of the milieu interne.[68] However, the condition is designated edema when an increase

in tissue fluid produces swelling of the area of structure More fluid enters than leaves the intercellular spaces[34] when the steady state is interrupted by osmotic mechanical or inflammatory changes Exudative edema fluid (exudate) usually has a high specific gravity, contains leukocytes and sometimes numerous erythrocytes and has a high level of plasma proteins and fibrin Transudates usually have a comparatively low specific gravity and contain much less fibrin and other protein Transudates occur as a result of increased capillary permeability, such as is common in cardiac failure[663] As early as 25 B C to 45 A D , Celsus recognized changes in the tissues involved in true inflammation, and used terminology which has stood the test of time so well that it is still employed[6 0] to designate the four cardinal signs In 1873 Samuel believed that the fluid in exudative edema resulted from changes in the walls of blood vessels[69]

Protein content and conditions that result in stasis, determine whether or not edema fluid can stimulate plasmacytogenesis The protein content of edema fluid is most commonly related to conditions favorable for the passage of the larger protein molecules through the capillary walls into a *favorable situation which provides a fibrin network to retain the infiltrated* lymphocytes and plasma protein Transudate such as that formed in cardiac or renal edema, is not favorable for plasmacytogenesis because it has a very low protein content and a much lower specific gravity than exudate[100, 663] The duration of the exudate and other factors activate plasmacytogenesis There may be formation of plasmacytes if a slight hyperemia exists over a long period of time whereas plasmacytes do not usually form when the same degree of hyperemia occurs for a short time

All investigators do not place as much significance on the part played by vascular disturbance in initiating inflammatory processes as the preceding discussion may indicate Ehrich[71] believes that the connective tissue ground substance is the site of the changes incident to initiation of inflammatory processes Dougherty[?40] supports the idea that the interstitial connective tissue is very important as a site of origin [01] Roessle suggested that inflammation is not necessarily dependent upon vascular changes [71]

PLASMACYTOGENESIS

The formation of plasmacytes in inflammation functions as a time bridge' mechanism whereby the increased protein that results from the first stage of inflammation is retained locally for later use by proliferative cells Part of the proteins from leaking capillaries as well as those from necrotic tissue and cytolyzed polymorphonuclear leukocytes or other blood cells are retained locally as components of the nucleoprotein that is synthesized in the cytoplasm of plasmacytes which form from lymphocytes

This time bridge' is a very important factor because the initial irri

tant, whether histamine serotonin or leukotaxin of Menkin[69] or some other similar substance, has usually disappeared before mitosis occurs in the regeneration or replacement of tissues Normally, hyperemia is controlled by hormones or neurones, but the chemically induced hyperemia occurs in earlier stages in the case of the healing of chronically inflamed tissues Transformation of lymphocytes into plasmacytes presents a form of local resynthesis and storage of ribonucleic acid (RNA) and globulin from protein that is released from necrotic cells and plasma protein to be utilized by other proliferating cells, such as fibroblasts in the case of scar formation Basically, this storage and retention of nucleoprotein is the same at sites of chronic inflammation as it is in those tissues where plasmacytes normally occur such as in the core of intestinal villi, in lymph nodes and the spleen[219] Lymphocytes and plasmacytes represent a form of protein storage, but these cells are not indispensable for wound healing by fibroblasts, epithelial and other cells

Knight[220] makes the statement that corns, calluses, etc , develop in areas where blood vessels are under pressure, and asks why the local anemia caused by this pressure induces these cells to grow more rapidly than other cells in this particular animal One possibility may be that a mild exterior pressure inhibits venous flow more than arterial flow and thereby causes partial to complete venous stasis without greatly inhibiting the arterial flow The end result would be the exudation of additional protein into the tissue fluid

Transformation of lymphocytes into plasmacytes does not occur in all lesions infiltrated by lymphocytes Some chronic inflammatory infiltrations are almost entirely composed of lymphocytes whereas most of the infiltrated cells in other inflammations are plasmacytes[7] Thus it is sometimes difficult to differentiate by histological methods alone between the tissue reaction to "the spirochete of syphilis and bacillus of tuberculosis [73] Ogilvie[7] suggests the following points as an aid in recognizing the differential characteristics Syphilis usually has a 'high proportion of plasma cells', whereas tuberculosis usually has a predominance of lymphocytes Vascular lesions especially pericapillary fibrosis are more common in syphilis than in tuberculosis Syphilis usually produces less caseous and more fibrous structure than tuberculosis Thus it appears that the preponderance of plasmacytes in syphilitic lesions is correlated with the increase in fibrosity, including pericapillary fibrosis and other vascular lesions whereas the preponderance of lymphocytes in tuberculous lesions is correlated with the relatively few vascular lesions and the pronounced caseous necrosis These comparisons lead to the conclusion that the amount of protein leaked from the capillaries and retained locally in the edema fluid is

insufficient in quantity or quality to promote transformation of lymphocytes to plasmacytes in tuberculous lesions [534]

SYMPATHETIC PARASYMPATHETIC BALANCE

Vascular changes similar to those in subchronic and chronic inflammation may be induced in certain structures by imbalanced stimulation of the sympathetic and parasympathetic innervations The syndromes in Mikulicz s disease Graves disease toxic thyroiditis and myasthenia gravis are usually characterized by extensive benign hyperplasia of lymphoid tissues in those organs where capillaries are subjected to extreme and intermittent changes in diameter and permeability, through stimulation by the sympathetic and parasympathetic fibers

Parasympathetic stimulation has the function of vasodilation in the lacrimal and salivary glands, the sympathetic has that of vasoconstriction [87] These glands are involved in Mikulicz s syndrome, [9] [54] which is characterized by hyperplasia of lymphoid tissues [54] However, some authors include hives, mumps lymphosarcoma and Hodgkin s disease as causing or contributing to Mikulicz's disease [965] The cause of Mikulicz s syndrome is generally conceded to be unknown Some writers point out that it is apparently due to infection [93] because 70 per cent of the cases have been attributed to staphylococcal infection [1059] Cameron[135] points out that obviously the etiology of this disease suggests a plurality of causes [54] [936]

Increased vasodilation of capillaries in the variously involved organs of Mikulicz's disease could be due to the decreased secretion of epinephrine or to increased secretion of acetylcholine In either event, the architecture and protein relations would be conducive to hyperplasia of the lymphoid tissues A number of case histories indicate clearly that degeneration of the cortex of the adrenal gland occurs in many of these patients The inadequate release of cortisone may cause hyperplasia of lymphoid tissues and lymphocytosis by decreasing the rate of lymphocytolysis

Lymphocytes and plasmacytes are present normally in the connective tissues of the salivary and lacrimal glands Also prolonged dilation and increased permeability of the capillaries produce an edematous condition in these glands which is functionally similar to the retarded flow of lymph in the sinuses of a normal lymph node or to chronic inflammation The loose connective tissues in these organs enables the proliferating lymphocytes to become organized into large areas of lymphoid tissues which may resemble the cortical regions of lymph nodes

Necrosis or faulty metabolism of neurons in the superior cervical ganglion could be a cause of Mikulicz s disease of the lacrimal and salivary glands and the benign hyperplasia of lymphoid tissue and the thyroid gland Some

tant, whether histamine, serotonin or leukotaxin of Menkin[68] or some other similar substance, has usually disappeared before mitosis occurs in the regeneration or replacement of tissues Normally, hyperemia is controlled by hormones or neurones, but the chemically induced hyperemia occurs in earlier stages in the case of the healing of chronically inflamed tissues *Transformation of lymphocytes into plasmacytes* presents a form of local resynthesis and storage of ribonucleic acid (RNA) and globulin from protein that is released from necrotic cells and plasma protein to be utilized by other proliferating cells, such as fibroblasts in the case of scar formation Basically, this storage and retention of nucleoprotein is the same at sites of chronic inflammation as it is in those tissues where plasmacytes normally occur, such as in the core of intestinal villi, in lymph nodes and the spleen [19] Lymphocytes and plasmacytes represent a form of protein storage, but these cells are not indispensable for wound healing by fibroblasts, epithelial and other cells

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a microorganism was retained However bacterial toxins and vital new red dye were forced through by filtration Thus it is suggested that barriers that function similar to cyprinine membranes might help to explain the failure of some localized foci to stimulate immunity It is probable that hyaluronic acid (HA) changes in the connective tissue ground substance can effect quite similar results [1] Lymph vessels proliferate in inflammation During the past 70 years [814] the proliferation of lymphatics has been repeatedly demonstrated, however Policard and Desplas believe that lymphatics are absent in the granulation tissue of secondary repair [814]

Fibrinogenesis

Fibrinogen a soluble protein escapes from the blood vessels in the exudate or by frank hemorrhage into the tissues at the site of inflammation trauma or other injury By a process variously held to be enzymic or stoichiometric [41] the soluble fibrinogen is converted into fibrin an insoluble protein by the addition of lysine and phenylalanine to the nine amino acids in the fibrinogen molecule [41] The percentage of these nine amino acids is identical within the range of the probable error of analysis in both fibrinogen and fibrin

Incorporation of phenylalanine occurs during the process of formation of fibrin from fibrinogen [1054] An excess of phenylalanine from any one of several sources over that required for fibrin formation may counterbalance the effects of histamine or serotonin on capillaries because phenylalanine can form tyrosine which in turn can be decarboxylated to form tyramine [1054] The formation of tyramine in areas of wound healing or in pathological tissues in which fibrin is being formed or at the time of fibrinolysis could constrict capillaries because it is a potent vasoconstrictor [1054] and thus counterbalance the effects of agents that cause capillary dilation Epinephrine may also control the trephocytic activities of the free connective tissue cells including mast cells

The causes of fibrin formation have been discussed in detail [41] however a specifically identified source of the phenylalanine and lysine which do not occur in the fibrinogen but are present in fibrin has not been revealed Phenylalanine and lysine occur in albumin gamma globulin and in the globulin of erythrocytes whereas lysine occurs in thymus histone [41] These two amino acids as free amino acids in tissue fluid or blood may be made available for the formation of fibrin from fibrinogen or from albumin globin lyzed erythrocytes or necrotic cells

The incorporation of lysine into fibrin may retain one of the indispensable amino acids in regions of inflammation and wound healing It is not known however if lysine is freed from fibrin and if so whether it is available for use by other cells Activated plasmin a primary cause of irreversi

of these conditions have been observed in Graves disease, toxic thyroiditis and myasthenia gravis The superior cervical ganglion of the sympathetic system contains the bodies of neurons which innervate the lacrimal and salivary glands[37] and, probably, the thymus and thyroid The extent of necrosis in the superior cervical ganglion and the resulting malfunctions would determine the extent to which lymphoid hyperplasia developed If the necrosis were limited to a very small area, only the neurons passing to a single gland or organ might be destroyed Consequently, vasodilation and lymphoid hyperplasia would occur in a delimited area or in only one of the glands Such differences have been described in Mikulicz's syndrome which sometimes is limited to only one of the salivary glands, but can occur in all of the salivary glands In this case, necrosis or dysfunction of neurons in a larger area would be affected Lesions in either the ganglia or the post ganglionic fibers would cause a decrease in secretion of epinephrine that could inhibit the constriction of capillaries and increase the vasodilating capacity of the parasympathetic fibers

FIBRIN IN REPAIR PROCESSES

The formation of fibrin in granulation tissue during wound healing localizes protein[9] by forming a network which increases the retention of plasma proteins as well as by serving as a deposit of available extracellular protein Fibrinogen, one of the three groups of plasma proteins, forms fibrin only under pathological conditions[60, 33, 633] Fibrin occurs as a protein clot sheet (membrane) or precipitate Fibrinogen circulates freely in the blood stream and forms about 7 0 per cent (0 43 g/100 ml) of the plasma protein as determined electrophoretically by Cohn and co workers in 1944[41] Fibrinogen is a long slender, fusiform molecule (about 33 by 900 Å) and has a molecular weight of 400 000 to 700,000[60, 412, 1094] Therefore, fibrinogen can escape in quantities from the blood only while the capillaries are in a state of greatly increased permeability or actually ruptured The amount of fibrinous exudate as indicated by fibrin formation varies greatly Little or no fibrin is formed by mosquito bites but much may form in the exudate of tuberculous lesions[633] Cantharidal blisters of human skin may be rich in fibrinous exudate[663]

The development of fibrin in granulation produces a screening effect that is, the protein molecules are retained within the fibrin network while the serum is permitted to exude through the granulation tissue[455] This viewpoint is especially significant because the increased retention of plasma proteins at this site creates a situation favorable for the transformation of some lymphocytes into plasmacytes Hughes[4] found that when a cyprinine "membrane was used as a filter it excluded trypan blue and certain other vital dyes from areas of inflammation and that anything as large as

cau e these cells decrea e as the proliferation of fibrocytes or other cells or the deposition of collagen replaces the area [534]

FIBROBLASTS AS A SOURCE OF AMP

Granules of mast cells contain AMP, but the primary and major source of *AMP is probably the fibroblastic connective tissue cells* [46] *Differen*tiated cells produce specialized AMP such as o sein and chondroitin sulfuric acid Fibroblasts are undifferentiated cells derived from mesenchymal cells[746] and are con_idered a source of collagen fibers [74 57] However the fibrocyte is a more highly differentiated form of this cell and is considered by others to be the ource of collagenous elastic and reticular fibers [59] Bensley [1] suggests that the ground ubstance may be derived from fibro blasts

The results of experiments with tissue cultures indicate that mast cells are not the only source of mucopoly acchandes (MPS) Fibroblasts in roller tube cultures produce collagen [4] In cultures of human exten or ten don of the toe the proliferating tendon cells produced MPS during the first 3 months after which the amount continually decreased [49] Cultures of *human skin produced a mixed poly accharide* apparently ⅔ of which was HA and about ⅓ chondroitin sulfate C [376] No mention was made of mast cells being present or absent in either culture of human tissue

Several characteristic properties of ground substance which Bensley [1] gives are the following It resists digestion with pepsin but is readily di gested with pancreatin It may be made to stain metachromatically with toluidine blue and it has an affinity for copper salts She takes advantage of this cuprophilia by mordanting in copper to obtain a deeper staining of the ground subst ance She found that connective ti sue from the cock s comb from areas of human skin that showed fibrosis resulting from car cinomatosis and from the mucosa of the human uterus all gave the same general positive results to tests for mucins

Other authors have beclouded the issue by attributing collagen forma tion to connective tissue cells without specifying which cells *are respon ible* Jacob on[476] states that collagen fibers are a characteristic feature of the connective tissues and their derivatives Kleiner[4] points out that all connective tissues contain mucoids Granules in fibroblasts have been con sidered as precursors of MPS in the ground substance but extracellular origin of AMP is also probable [10 7] Jacobson[4 6] points out that the inter cellular spaces are filled with matrix and fibers but that it has been im *possible to establish an intr icellular origin of their precursors*

The more highly differentiated cells which arise from fibroblasts or mesenchymal tissue produce AMP However a review of the relations of the e more specialized cells to the formation of AMP indicates that the

ble disintegration of fibrin, occurs in blood, tissues and organs, especially in the lungs and adrenals It is normally inactivated by antiplasmin[6 6] *Lysine must be supplied as a ready made whole molecule in the diet*[60] because it cannot be synthesized Rose (1938) believes that lysine is one of the three amino acids which have been definitely proved to be indispensable[1094] Schoenheimer[908] used radioactive nitrogen for the "amino group and deuterium for the carbon chain" He found that lysine can contribute 'nitrogen for the formation of other amino acids but that it was the only amino acid which is unable to accept nitrogen from other amino acids' *Thus, lysine is considered to be a single, indispensable, chemical unit*[908] Additional significance might be attached to the incorporation of lysine in fibrin, its retention in the area, and subsequent availability during the process of fibrinolysis

Fibrin and Plasmacytogenesis

Fibrin formation causes partial stasis of lymph and tissue fluid within the network of fibrin fibers This network has irregularly sized meshes and helps to confine lymphocytes, extravasated erythrocytes and other cells, as well as the plasma proteins, to the area of the exudate or frank hemorrhage[33 534 6 6] Partial lymphatic occlusion also favors the retention of cells and proteins

Fibrin is first laid down as a meshwork of fibers which becomes increasingly dense until a fairly solid matrix is ultimately formed[33] Ferry (1948) believes that when fibrinogen is changed into fibrin, the network of fine *strands is formed by end to end union of the molecules which are cross* linked at irregular intervals by primary chemical bonds[790] This fibrous structure serves as a support for the injured tissues At the same time, it causes a certain amount of lymph stasis in the tissue and lymph capillaries. By this means, fibrin confines proteins and lymphocytes in the exudate, thus facilitating plasmacytogenesis the deposition of collagen, and wound healing

The transformation of lymphocytes into plasmacytes follows the retention of protein during the first stage of granulation tissue formation Apparently, the formation of plasmacytes is concerned with initiating regeneration and repair However the necrosis of tissue cells dissolution of blood clots exudation of proteins from blood capillaries and proteins from lysis of polymorphonuclear leukocytes and monocytes also increase the proteins for the transformation of some of the lymphocytes into plasma cytes Lymphocytes and plasmacytes as well as the polymorphonuclear leukocytes and *monocytes have been credited with preventing infection* of the exposed surface of granulating tissue[10 9] However the synthesis and retention of nucleoproteins in these cells may be a more basic function be

cause these cells decrease as the proliferation of fibrocytes or other cells, or the deposition of collagen replaces the area [534]

FIBROBLASTS AS A SOURCE OF AMP

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more specialized cells may obtain AMP or its components indirectly from the connective tissue ground substance Mast cells do not appear to be precursors of these varied forms of intercellular AMP, as indicated in sectioned tissues or as evidenced in embryological development

The formation of AMP by osteocytes gives an illustration of the complexity *in determining the origin of various AMP* *The osteoblasts* have been assumed to deposit bone matrix, but the intracellular precursors have not been identified[290] Heller Steinberg states that periodic acid Schiff (PAS) positive granules are more abundant in osteoblasts in growth zones, and are considered precursors of bone matrix-[26] Bone contains several forms of AMP, including ossein, which is similar to collagen in other connective tissues, osseomucoid and osseoalbuminoid[39 633] The organic portion of bone comprised 30 per cent of the matrix in contrast to the inorganic portions which comprise 60 to 70 per cent[39]

The osteoblasts obtain AMP from the primitive fibrous tissue, whereas the osteoclasts obtain it from pre existing cartilage in bones formed by endochondral ossification Sylven suggests that the chondroitin sulfuric acid protects the cartilage against calcification because it is acid and would inhibit the calcifying action of alkaline phosphatase He observed a loss of chondroitin sulfuric acid prior to the calcification of cartilage, which he attributed to a change in pH or enzyme action[393] However there is the possibility that here again as in the case of odontoblasts, the osteoblasts are utilizing AMP that thas been formed by other cells for the synthesis of ossein, osseomucoid and osseoalbumin[407]

Determining the origin of the various forms of AMP in bone, cartilage and dentine is complicated In the case of bone the synthesis of AMP begins with mesenchymal cells depositing AMP in intercellular material, followed by the utilization of these AMP or their components by chondroblasts in the case of cartilage and finally the utilization of chrondroitin sulfuric acid of cartilage by osteoblasts in case of bones formed from cartilage This series indicates the possibility that intercellular substance may provide trephones for the synthesis of AMP and may account for the failure to determine the relation of osteoblasts to the formation of the AMP in the matrix of bone The above series of changes may be reversible and thus account for the 'turnover' of AMP Slack[849] studied the replacement of collagen and reported that the turnover was highest in collagen from bone However the turnover rate of all collagen was indirectly proportional to the age of the rats but in all cases the turnover was low compared with intercellular protein

Enamel of teeth contains metachromatic AMP[900] and mucoprotein[811] Dentine contains chondroitin sulfuric acid[801] or collagen[97] Ameloblasts and odontoblasts may synthesize AMP from components but these cells

may draw upon the AMP which has been de cribed by Hamilton and Koning[393] to be 'present as the fibrillar matrix in the intercellular spaces of the pulp at all developmental stages Vitamin C deficiency causes a failure of odontoblasts to deposit dentine and the formation of abnormal or decreased collagen by fibroblasts The effect of vitamin C deficiency may be mediated directly or indirectly through abnormal collagen formation by fibroblasts

Corneal corpuscles which are fixed flattened cells lying within the lamellae of the substantia propria (third layer) of the cornea and the collagenic fibers are primarily responsible for the deposition of 'true hyaluronic acid sulphate which forms the amorphous cement like substance within the substantia propria and thus is largely responsible for the unique transparency of the cornea of the eye[393, 865] However Ham[393] admits that the arrangement of the collagenic fibers and fibrils in the substantia propria may contribute to the transparency of the cornea He points out that nowhere else in the connective tissue of the body has true hyaluronic acid sulfate (unimolecular units of acetyl glucosamine glucuronic acid and sulfuric acid) been recovered It appears that MPS play a very important part in corneal transparency

Effect of Hormones

Changes in the proliferation of fibroblasts follow stimulation by several hormones The formation of ground substance (and its methchromasia) and hydration increase in the process of widening of the symphysis pubis in the pregnanat guinea pig[86] This change is attributed to the relaxin effect found by Hisaw[438] in first guinea pigs and later in the 13 lined spermophile Silberberg and Silberberg[937] administered estrogenic hormones to young adult guinea pigs deprived of ascorbic acid The estrogens greatly modified the usually scorbutic sequences especially by counteracting hemorrhage edema and bone resportion In old guinea pigs whose skeletons had practically matured before they were deprived of vitamin C the chief effect of the hormones was to decrease hemorrhage and periosteal edema and to produce a slight increa e in density of the bones The beneficial effects of the estrogen treatment were attributed chiefly to hyalinization of the connective tissue Also estrogen caused excessive deposition of os eous tissue in the marrow cavity and interfered with bone resorption and apposition in otherwise normal young growing mice[934]

The increase in mast cells in the uterus of hamsters after the administration of estrogen may represent a direct source of AMP or an indirectly available ource through the action of histamine and the increase of capillary permeability [9] In the ame manner that we have described the infiltration of lymphocytes and plasmacytes as a time bridge for protein

the formation of mast cells in the mammary gland and uterus[5 9 530] may represent a "time bridge" for heparin and histamine In addition, Compton[181] reported that many mast cells are present in the basal layer of the endometrium around the uterine glands and in the myometrium of hamsters Also, he mentioned a possible relation of mast cells to menstrual bleeding and the non coagulation of menstrual blood

Replacement Processes

Fibrocytes produce AMP in many pathological replacement processes The fundamental alteration in collagen diseases is the proliferative, degenerative and necrotic changes in fibroblasts which accompany the development of swollen mucoid ground substances [83] Chrondoblastoma fibroblastoma, myxoblastoma, osteoblastoma and certain normal situations *contain fibroblasts which may produce mucin, chondromucin and osseomucin,"* as well as collagen and elastic fibrils and/or fibroglia [633] Gingival fibromatosis in open and closed ulcerative pulpitis causes the deposition of collagen by fibroblasts and fibrous tissue [996]

The increase of mast cells in some pathological conditions is not sufficiently great to represent a major or actual source of AMP in amyloid or mucoid formation in those conditions in which MPS are increased The primary source of AMP in amyloid formation is probably the fibroblast but mast cells may play a contributing role in amyloid formation which may be caused indirectly by the effects of mast cell products on capillaries Angevine[14] calls attention to the fact that the formation of AMP in many types of inflammation is correlated *with glycolysis Malignant fibroblasts* produce varying amounts of collagen and known MPS Some fibromas such as certain fibrosarcomas contain very little intercellular material whereas other tumors such as certain myxomas and keloids are composed predominantly of intercellular material [633]

Experimentally induced changes in the number of fibroblasts alter the amounts of collagen that are deposited Ingle and Baker[464] state that cortisone inhibits the formation of chondroitin sulfate by injuring the capacity of fibroblasts to form ground substance Clark and Umbeit[10] *review the* effects of cortisone on decreasing synthesis of chondroitin sulfate in granulation tissue, and in the synthesis of chondroitin sulfuric acid by embryonic and wound tissue *in vitro* Scurvy is attended by a decrease in the number of fibroblasts and the formation of collagen [97 407 61]

Collagen in Wound Healing

Collagen plays a conspicuous part in wound healing particularly in the formation of the cicatrix and as an intercellular cementum Recently as

a re ult of the work of Asboe Hansen and colleagues considerable interest has been manifested in the possible relations of mast cells to collagenesis in arthritis and collagen diseases as well as in wound healing It has not been determined definitely if mast cells function in the formation of collagen by supplying certain MPS directly or indirectly by producing sustained, increased capillary dilation and permeability Studies on fibroblasts as a source of MPS have hown that fibroblasts form collagen There is evidence that the mast cell is both directly and indirectly concerned with collagenesis However the general principle of the relationship of mast cells to local increase in tissue fluid and plasma borne nutritive substances has not been acceptably established, and the method by which collagen forms has not been determined Jackson[473] considers numerous theories including Bait sell s (1915) idea of the conversion of blood plasma fibrin into collagen, and Gruyon s (1934) hypothesis that collagen is derived from fibrinogen Chemical analyses, as explained by Astbury (1947) have established that fibrin belongs to a different group of proteins which includes keratin myo cin and epidermin [4 3] Thus, the origin of collagen remains an open question

The little appreciated versatility of mesenchymal cells as the primary source of both mast cells and collagen, and the presence of AMP producing fibroblasts, probably are impediments in determining the relation between mast cells and collagenesis This point of view becomes more significant when viewed in the light of the common observation that both mast cells and collagen are rare in several structures and organs The endometrium, parenchyma of the central nervous system, suprarenals and kidneys are practically devoid of mesenchymal stem cells necessary for the develop ment of mast cells fibroblasts and possibly other controversial cells

There are two other points to be considered There is evidence that the crest in mast cell population has pa sed before a notable increase in col lagen occurs as was observed in the mammary gland of hamsters receiving estrogen [530] Furthermore mast cells probably resynthesize AMP from the components of degenerating collagen Signs of degeneration of the increased collagen herald an increase in the number of mast cells which appear to be salvaging and storing materials freed from the degenerating collagen Under these conditions the mast cells would be important as a temporary storage of AMP rather than having a causative effect For example it has been ob served that fresh lesions of disseminated lupus erythematosus (one of many collagenous diseases) showed mast cells and hyaluronidase sensitive metachromatic connective tissue ground substance Older lesions however had a reduction in metachromasia and respon e to hyaluronidase and frag mentation and degeneration of collagen fibrils [19] However there were in filtrations with lymphocytes mast cells and plasma cells [1 19]

NATURE OF COLLAGEN

The nature and functional relations of collagen are extremely diversified Robb Smith[864] points out that it is impossible to be certain whether the samples of collagen used in various methods of investigation, such as chemical or x ray diffration, would be recognized as collagen by the histologist The importance of collagen is indicated by Gustavson,[38] who states that about one third of the total mass of proteins in the body is collagen Collagen, usually reinforced by a certain amount of elastin, forms by far the major part of the intercellular substance which binds or cements cells together [393] This is especially noticeable in certain epithelia, such as the shed epidermis of salamanders and frogs, and is always present as the basement membrane connecting epithelia to connective tissue [393] Distinction between the two kinds of fibers is supposedly easily made because collagen is PAS negative and reticulin is PAS positive [60] However, in attempts to determine whether pseudoxanthoma elasticum is a dystrophy of the collagenic or elastic fibers in the skin, indications were found that the elastase in this material probably acted as a mucase by dissolving the mucoproteins[671] that bind the components of molecules and fibers together to form collagenous tissue [90, 130, 38] In skins and hides collagen forms 90 to 95 per cent of the solid found in the corium [70] and about 72 per cent of the total dry weight of normal human skin [671]

Collagen changes with age Neuberger and colleagues (1951, 1953) show that the metabolism of rat tendon collagen is more rapid in young than in old animals as indicated by the gain and loss of isotopicglycine [9 0] The solubility of collagen in acetic acid also progressively decreases with age [9 0] Collagen is relatively inert metabolically at all ages but the total amount increases with age [9 0] However it is doubtful if advanced age in itself causes a notable increase in the total amount of collagen Kellgren (1953) believes that *instead of a collagen turnover a formative process and* a destructive process may be operating independently [8 8] Probably collagen has little importance as a source of protein because of its insolubility

CHEMICAL PROPERTIES

Formerly collagen was called ossein [367] It is an albuminoid (scleroprotein) substance[407, 630] and is composed essentially of several mucopolysaccharides combined with a protein of unknown nature [387] Polysaccharides are present in collagenous tissues (1 28 per cent glycoprotein in ox tendon of Achilles) [419, 1084] and their sulfate binds the fibrils together [47] MPS forms part of the collagen molecule [99] is related to the determination of the length of the internodes in fibrils,[39] and is essential in the maturation of collagen [130]

Collagen contains much less *sulfur than keratin* [41] *The sulfur content*

of hide collagen (presumably bovine skin) should be about 0 15 to 0 2 per cent while collagen from the middle layer of the dermis contains about 0 3 per cent[38] These volumes are based on the tenet that the only sulfur-containing amino acids in the collagen molecule are methionine[38] and cystine[40]

The total nitrogen value of the 21 amino acids found in extracts of collagen is about 18 6 per cent[38] About one third of the total amount of *amino acid residue in the collagen molecule has been estimated to be glycine*[41] Proline and hydroxyproline represent another third of the molecule and the remaining third is made up of other amino acids[41] Hydroxyproline is probably the most characteristic of the amino acids in the various types of collagen[38] In contrast to histones collagen contains a low percentage of most essential amino acids and does not contain cysteine methionine tryptophane or tyrosine[38,9] However it is subject to some alteration because the total amount of collagen and the degree of orientation of collagenous structures tend to increase with age

The minimum molecular weight of collagen is about 39 000 as determined from the amino acid distribution The real molecular weight is indicated as being in the millions with about 10 million having been suggested for acid extracted skin collagen[38] The isoelectric point of collagen appears to be related to the source and treatment given the material to be extracted and the method of extraction of the sample It has been shown that the isoelectric point of native hide collagen ranges from pH 7 0 to 7 8 but for limed (alkali treated) hides presumably of cattle it is shifted to about pH 5 3[38]

Collagen differs in various species of animals[19, 38, 410] and in various structures in an individual[39, 37, 38] under different conditions of age or nutrition[39, 89, 38] and as a result of different conditions of extraction[38, 89, 38, 410, 908] Extracted collagen is also affected by the degradation of the native collagen[38, 410, 8, 8, 905]

COLLAGENASE

Collagenase is a term applied to a proteolytic enzyme considered by Maschmann (1937 1939) *in his description of proteases that were obtained from filtrates of Clostridium welchii* and three other species of Clostridium[863] The term collagenase actually includes a group of collagen destructive enzymes the most potent preparation of which is a mixture obtained from *C histolyticum*[38]

Several enzymes alter collagen in one way or another but it is sometimes difficult to determine the mode of action of the enzyme Neuman and Tytell (1950) believe that native collagen is attacked only by pepsin and certain enzymes *of C welchii and C histolyticum*[863] Harrow (1950) states that

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sue.[38] Investigators do not agree on the nature of native collagen[38, 387, 4 1] or on its mode of development to form collagenic fibrils.[451, 510] A valid reason for this disagreement may be that almost anything done to native collagen, including dehydration[38, 8 8, 863] and hydration,[38, 863] alters it. Also, it is well known that boiling in water converts collagen to gelatin.[407, 1094] Thus the manner of preparation of the collagen sample may be reflected in the results of its chemical analysis. Jacobson[476] states that 'nothing is known of a specific factor inducing the formation of either collagen or elastic fibers, which are histologically very similar in embryonic and granulation tissues.[560] However, Kulonen[570] points out that recent investigations employing electron microscopy and x ray diffraction have revealed the composition of collagen, but work on the 'arrangement of the amino acids in the polypeptide chain remains in the progressive stage. Other investigators have been able to produce elastin from prepared collagen and by the use of the enzymes collagenase and elastase, and electron photomicrography to show that structurally collagen in young individuals is composed chiefly of elastin.[510]

Long before the advent of the electron microscope, collagenous fibers and bundles of fibers were recognized to be white, or collagenous, connective tissue in cartilage, fasciae, ligaments, tendons, skin and other structures—and to impart inelasticity and strength.[38, 393, 476, 746] White fibrous connective tissue contains a high percentage of collagen (31.6) for bovine *tendon of Achilles.*[41, 1094] *Whereas white fibrous connective tissue is a* collagenous tissue, it should not be considered collagen because it contains about 4 per cent of other proteins.[1094]

Collagenous fibers are the most prevalent of three types of fibers (collagenic, reticular and elastic) found in the connective tissues.[476, 746] Also, collagen is present in the connective tissue of peripheral nerves. Abercrombie and Johnson (1946) report that peripheral nerves of the rabbit contain 3.2 per cent collagen, and a similar value has been reported for the peripheral nerves of the cat.[881] However, collagen does not occur in the central nervous system[407, 881] except in the walls of blood vessels, as has been demonstrated by histochemical methods.[881]

Skin is rich in collagen, and most of the collagen used in chemical research is extracted from hides.[38] Few attempts have been made to correlate the mast cell population of the skin with collagen content or with age changes in man or mammals. However, Hill and Montgomery (1940) examined skin specimens from unexposed regions of the body of 20 autopsied people ranging in age from newborn to 78 years. Ejiri (1936, 1937) examined 718 skin samples of various ages taken at autopsy from exposed areas of different parts of the human head, forearm and back of the hand. Each of these investigators reported finding minimal age changes in the

pepsin fairly well digests collagen, but that trypsin has only a slight effect on it [38] Denatured or swollen collagen is much more readily split by tryp sin and other proteinases than is native collagen which is practically non susceptible to proteolytic enzymes unless swollen or otherwise denatured [38] Collagenase is specific for collagen, but Robb Smith[863] cautions that col lagenase "is not available in anything like a pure state" He further points out that the "collagenase of Clostridium toxin, prepared from filtrates of the anaerobic bacteria belonging to that genus is very impure so that "one has no real idea what other enzymes may be present in a bacterial filtrate" This statement is supported by the finding that *C welchii* Type A filtrate contains appreciable amounts of lecithinase, hyaluronidase and several other substances which have ill defined biochemical and biological char acteristics [863]

Denatured collagen is more readily split by proteinases than is native collagen which is scarcely affected by proteolytic enzymes unless it has been denatured Proteinases of the cathepsin group do not have any action on collagen *in vivo* because these enzymes require a pH of about 5 [38] Thus proteolytic enzymes have little or no effect on native collagen unless it is subjected to conditions such as are produced by the addition of hydro chloric acid which hydrolyzes and loosens the fibers, or by the addition of other agents which cause swelling of the collagenous material [38]

Gustavson[38] points out that unsuitable substrates such as reconstituted or denatured collagen preparations have been used instead of native col lagen in most of the investigations of collagenase activity 'Thus Na geotte s 'collagene A paper (paper impregnated with acid extracted col lagen) and 'Azo coll (hide powder coupled with certain azo dye tuff) have been used in the majority of investigations [38]

The numerous forms of Clostridium are anaerobic spore bearing, gram positive rods [39] and some if not many of them are capable of producing collagenase[38, 863] and gas gangrene [39, 38, 863] Thus numerous investi gators believe that the ability of these organisms to produce collagenase intimately connects them with gas gangrene and collagenous diseases [38, 863]

Collagenase is specific for collagen but it has been pointed out that as yet it is not available in anything like a pure state the confusing results obtained by using *C welchii* toxin or other bacterial filtrates probably in dicates that enzymes other than collagenase are also present [863] Obviously the use of collagenase will be limited as an aid in identifying collagen until this enyzme is available in pure form

PHYSICAL CHARACTERISTICS

Collagen is an amorphous essentially free substance in the tissue fluid It is considered to be a constituent of ground substance in connective tis

vealed by the electron microscope and by various applications of x ray diffraction and other means[38 8 9]

The terminology meaning and connotation of the terms used by various individuals investigating the origin of collagen are little short of bewildering A Neuberger introduced this problem on semantics for discussion by members of the Faraday Society in a meeting at King s College London in 1953 Members of the group, designated as Discussion G considered the question at some length Apparently although all disliked the use of the term "procollagen" they discussed standardization of the use of substitute and other terms including collagen precursor alkali soluble collagen acid soluble collagen, extracted collagen and collagen A—but took no action[8 8]

POLYSACCHARIDE IN COLLAGENESIS

The concensus as previously indicated appears to show that collagen is essentially protein in nature and that it is held together by an interfibrillar AMP cementum If this is the case the direct relation of mast cells to collagenesis is that these cells may supply the AMP[7 5 8 9] to bind the collagenous fibrillae and fibers together to form collagenous tissue It has also been suggested that the AMP may form part of the collagen molecule[99] and that it is essential in the maturation of collagen[130] The indirect relation of mast cells to collagenesis is related to their ability to produce hyperemia and increased capillary permeability with protein leakage chiefly by the release of histamine and heparin If the serotonin that is released from mast cells plays a role in collagenesis it would be expected to function through its vascular effects Ham[303] suggests that the polymerization of a formless precursor substance probably in the presence of an unknown influence exerted by living fibroblasts as a prerequisite is responsible for the formation of collagenic protofibrils and fibrils

It has been suggested that the formation of collagenic fibers may involve both soluble protein and a MPS Just how the two fractions are associated is not clear[99] Bowes and colleagues[99] state that dispersion involves the dissociation of the MPS fraction which would implicate a reverse process Other investigators have presented strong evidence showing that AMP (supposedly chondroitin sulfate) functions as a stabilizing agent in the process of converting soluble to insoluble (mature) collagen[130]

Bowes and Kenton (1950) and Partridge (1948) believe that the interfibrillar cement which holds the collagenous fibers together is probably chondroitin sulfate or that it is a similar AMP[4] Jackson[47] relates the cross linking of collagenic fibers to this AMP cement substance and believes that AMP is possibly important to the stability of connective tissue and that this relationship may be a significant factor to be considered in the pathogenesis of collagen diseases

dermal collagen from the various exposed and unexposed areas of the human subjects, but they did find a definite age correlation in fragmentation and degenerative changes in the dermal elastic fibers.[187] The work of Sundberg[99] on the mast cell population in the wall of the aorta, vena cava, subclavian and external iliac veins of 145 autopsied bodies indicates that there is not a well defined correlation between the reduction of mast cells and advanced age in man.

Formation of Collagen

There are various theoretical explanations of the origin of collagen, and many explanations of its chemical structure.[39, 8, 9, 917] However, comparatively little is definitely known about the origin or the chemical structure of native, as opposed to extracted, collagen. Numerous investigators have employed nearly every known method of approach to the solution of these two perplexing problems, as well as to the solution of problems related to the chemistry of the development, nature and environment of native collagen and the reconstitution of collagen fibers from extracted collagen.[39, 99, 38, 474]

One cause of difficulties in determining the origin and nature of collagen is that extracts of formed collagen are often impure.[8, 9, 863, 949] It is almost impossible to do anything to native collagen without degrading it.[8, 8, 863] *In addition, there are many kinds of collagen in various structures of a single organism.*[99, 3, 39] Furthermore, collagenase, the supposedly specific enzyme, is not available in a pure state.[863] Thus, these factors and other conditions indicate that the investigation of the origin and structure of collagen presents the familiar problem of how to interpret unknowns in terms of unknowns.

It has been known for several years that collagenic fibers and bundles of fibers may be disintegrated by dilute acetic acid to form a colorless viscous fluid and that collagenous fibrils may be re formed by treating this viscous fluid under proper conditions and with the proper amount of sodium chloride.[99, 38, 393, 0] These reconstructed ultramicroscopic fibrils show the same cross banded structure with varying intervals between the striations, and otherwise resemble native collagenic fibrils when compared under the electron microscope.[99, 393, 906] However, if the acid solution is heated, striated fibrils cannot be obtained from the degradation product[9, 6] because heat changes collagen to gelatin. When hydrated by boiling in water, tough collagenic fibrils and bundles are changed into *amorphous* gelatin.[393, 5, 0] In short, it appears that much of the available information on the formation of collagen is based upon deductions drawn chiefly from work concerned with the dissolution of collagenous connective tissues and reconstitution of the characteristic cross banded collagenic fibrils as re

are collagen precursor,[8 9] collagen or collagenic protofibrils[393 906] pre-precursor substance[393 8 8] procollagen,[407 906 950] protofibrils[393] and tropocollagen[35 906]

Procollagen Procollagen was so named by Orekhovitch (1950) who implied that "it is a precursor of insoluble collagen and also stated that, although never abundant it occurred in the connective tissue of young animals in the greatest amounts and progressively diminished with age[9 0] The procollagen of Orekhovitch and co workers (1952) is an acid soluble protein resembling collagen[40] It is a fibrous precipitate obtained by using a citrate buffer at pH 3 5 in extracting the connective tissue[906] However this tenet has been somewhat shaken by the turnover studies by Harkness and co workers[40] of the incorporation by skin collagen fractions of alpha C^{14} labeled glycine administered to rabbits[906] Neuberger and others (1953) object to the use of the term procollagen because it presupposes that a particular collagen fraction is actually a precursor of insoluble collagen[8 9]

Tropocollagen Schmidt Gross and Highberger (1955) used the term tropocollagen to designate a parent collagen that was composed of protofibrillar particles that had lengths of 2000 Å or more which they obtained by dialysis of extracts of collagenous connective tissue They suggest that tropocollagen is synthesized in the cells and that it is later converted into the characteristic fibrous form in the ground substance[39] Boedt and Doty (1955) determined that tropocollagen consists of rod shaped particles that have a length of 2900 Å a diameter of 14 Å and a molecular weight of about 300 000[38]

Schmidt Gross and Highberger[906] dialyzed a solution of ichthyocol and alpha acid glycoprotein From the filtrate they obtained electron photomicrographs (× 20 000) showing elongated tactoidal particles which they suspected of being composed of only a few trophocollagen particles This suggests that possibly the next step in the formation of collagen fibrils from tropocollagen would be expected to be the aggregation of a few tropocollagen particles to form the elongated tactoidal particles

Protofibrillae Ham[393] suggests that polymerization of a formless precursor substance, probably in the presence of an unknown influence exerted by living fibroblasts as a prerequisite is responsible for the formation of collagenic protofibrils In the structure of collagen protofibrils are considered by Bear (1952) to be linear aggregations of 12 by 640 Å, each of which may indicate the domain of a collagen molecule these protofibrillae are believed to be parallelly packed to form the collagen fibril[906] There is no apparent differentiation between Bear s term protofibril' and Gustavson s[38] or Schmidt Gross and Highberger s[906] tropocollagen

Ascorbic Acid Deficiency of vitamin C results in defective wound healing in soft and supporting tissues because there is interference with the

A comment on the histochemistry involved may be in order at this time Jacobson[476] points out that it is impractical to stain precursors of collagen by the Hotchkiss (PAS) method, for the more soluble material is dissolved out Also, it is impossible to see precursors of collagen in sections or in tissue culture with an optical microscope[8 8] It is claimed that the distinction between collagen and reticulin is readily made Collagen is PAS negative, whereas reticulin is PAS positive[560] Both water soluble and water insoluble AMP are PAS positive[709 5 9 530] Collagen stains readily with aniline blue by Mallory's aniline blue method,[09 530] but it is said to be PAS negative[516]

PRECOLLAGEN

Most attempts to identify precursors of collagen have been made by analysis of degraded collagen which was obtained from extracts of skin[38] *or rat tail tendon,*[10] *or by studying the reconstitution of collagen from* such extracts[99 38 570] Harkness and colleagues (1954) incorporated alpha C^{14} labeled glycine into soluble collagen that was extracted from the skin of young rabbits and they obtained three forms of collagen by varying the extraction procedures[950] One of these forms which was obtained by extraction with acetic buffers, such as citrate pH 4 0 was found to be essentially the same as the soluble collagen of earlier French workers and to be identical with Orekhovich s (1950) procollagen[950] The other two were identified as an alkali soluble collagen and "mature" insoluble collagen Each of these collagen fractions had a well defined individual characteristic mode of incorporating the labeled glycine[950] The reconstituted fibrils from these collagen fractions as well as from those obtained by Schmidt and co workers,[38 906] are readily identified with the electron microscope by *the distance between the cross striations The fibrils of one fraction have* intervals between the striations of 640 Å as in native collagen A second type of fibril fibrous long spaced" (FLS) has intervals of axial periods of 2000 to 3000 Å the third type segment long spacing' (SLS), has ' axial periods several times that of native collagen and other characteristics[906]

The substance which would meet all requirements for a precursor of collagen has not been identified Jacobson (1953) points out that precursors cannot be seen in cultures or sections with the optical microscope and that preparation of tissues for differential staining especially for PAS treatment which does not stain collagen[56?] would remove the more soluble material[8 8]

The concept of an unknown, early pre or pro substance being the source of collagen is responsible for the introduction of several seemingly synonymous *terms each of which was apparently considered by its sponsor* to have a special connotation Some of the terms used in this connection

genic cells of the chick in three types of guinea pig plasma. The cells cultured in plasma from scorbutic pigs formed very few fibers. Cells cultured in plasma from scorbutic pigs which had been treated with large doses of ascorbic acid for several days before the serum was obtained and cells cultured in scorbutic plasma to which ascorbic acid crystals were added formed fibers as rapidly as in normal plasma.[99]

Dentine, a polysaccharide which contains a sulphate of hexoamine,[79] does not develop in the absence of ascorbic acid. Absence of vitamin C causes abnormal mitotic divisions and the formation of large abnormal nucleolar masses containing ribose polynucleotides in the odontoblasts and hemorrhage in the pulp of the teeth.[956] These effects are partly explained by the fact that dentinal protein is collagen[4, 5] and that ascorbic acid controls the function of mesenchymal cells[594] which form the collagen fibers in the organic matrix of dentine. Fortunately, it is claimed that human teeth are not as susceptible to the effects of insufficient ascorbic acid as are the teeth of experimental animals.[164] Wolbach and Howe suggested that scurvy prevents jell formation in the teeth, as evidenced by the pulp being separated from the dentine in sectioned human teeth. Ham and Elliott[334] however believe that this conclusion may be based upon an artifact.

Ascorbic acid has miscellaneous functions in the normal metabolism of several AMP. It may function in the synthesis of glucuronic acid which is a component of most AMP. Also vitamin C may be involved in the synthesis of AMP that are polymers of acid sugars[416] and it may function in the polymerization of AMP as indicated by a lower degree of polymerization of these polysaccharides in the ground substance of scorbutic guinea pigs.[319]

Small amounts of ascorbic acid are required for normal metabolism. All animals except guinea pigs, monkeys, apes and man are able to synthesize ascorbic acid, presumably from glucose.[8] Wells[1151] states that metachromasia appears in the ground substance and that other processes, such as vascularization and fibrillogenesis, become normal within 6 to 12 hours after giving vitamin C to scorbutic guinea pigs. In addition, ascorbic acid is probably not a precursor or a component of collagen, as indicated in studies in which carbon labeled ascorbic acid was injected into guinea pigs. The radioactive ascorbic acid was partially excreted in the urine as calcium oxalate but most of it was eliminated in the form of carbon dioxide.[719]

The beneficial effects of ascorbic acid in some conditions of wound healing may be due to functions in regulating capillary permeability and to a function in detoxication. Ascorbic acid has been considered to maintain the balance between the so-called spreading factor (hyaluronidase) and HA and to maintain proper conditions in the cement substance of blood capillaries[55] and in the perivascular ground substance.[90] This function would explain the increased vascular permeability which Imagawa[460] describes

formation of fibroblasts and collagen Vitamin E is also necessary for the formation of collagen and consequently for the proper healing of wounds [38] The histogenetic sequence of the functions of vitamin C is established [1006, 1074] The absence of ascorbic acid results in scanty reticulin and the failure of fibroblasts to develop collagenic fibrils and to produce extracellular metachromasia, which is indicative of amorphous connective tissue ground substance The absence of ascorbic acid *affects bone matrix, dentine, carti*lage non epithelial cement substances fibrous tissues, oral mucosa,[164] muscle fibers, eyelids conjunctivae, heart, serosa (by ascites), lymphatic tissue and adrenal glands [90] Vitamin C deficiencies greatly reduce or even destroy binding and supporting connective tissues in both young and adult experimental mammals [8, 96, 394, 407, 715, 746, 83, 1084] This condition responds to the addition of ascorbic acid to the diet [78, 746, 83]

The relations of ascorbic acid to repair and growth are so far reaching that minor deficiencies may have surprisingly deleterious effects on collagen formation Lack of vitamin C has been reported to cause the dis*ruption of healed wounds and fractures of many years standing* [103, 1006] Ham and Elliott[394] suggest that ascorbic acid deficiency does not accelerate destructive processes but does cause failure in the formation of new connective tissue [61] The callus of previous fractures may be partly resorbed and again becomes the site of fracture when animals are subjected to a diet that is deficient in vitamin C [78]

Ground substance Connective tissue ground or intercellular, substance receives and stores the crude or basic materials for maintaining the normal connective tissues and for the formation of scar tissue in wound healing The absence of ascorbic acid is first manifested in the ground substance and subsequently in the formation of new connective tissue Obvious effects of scurvy in tissues are attributed to the failure of the connective tissue to form new connective tissue cells and the failure of the formation of intercellular cement such as the basement membrane of epithelia [394] It has not been established whether defective wound healing in scorbutic guinea pigs is 'in extracellular gellation or in the production of a precursor or factor by the connective tissue cell [610] The use of radiosulfate and radiophosphate *indicates that scurvy reduced and produced* an accumulation of polymerized products and that it diminished the formation of sulfomucopolysaccharide [319] Procollagen is reported by Orekhovich (1950) to be lower in scorbutic than in starved or normal animals [83] and to be the part of the ground substance which is most strongly affected in avitaminosis of vitamin C

Tissue culture methods indicate that vitamin C has a function in AMP metabolism *Querido and Gaillard (1939) demonstrated* the dependency of collagen formation upon the presence of ascorbic acid by culturing osteo

animal Swanson, Sigal and King (1936) found that increased ascorbic acid has a protective action against the effects of diptheria toxin on the teeth [594] Damage of dentine and sometimes of enamel by diptheria toxin is proportional to the degree of avitaminosis C Usually this damage may be prevented by administering increased amounts of ascorbic acid [594]

Function as hydrogen acceptor Ascorbic acid may function as a hydrogen acceptor in the normal metabolism of AMP Several relations indicate that ascorbic acid is important in the oxidation of glucose to glucuronic acid It has been established that hydroascorbic acid is readily reduced by glutathione [1084] Reversible oxidation reduction between ascorbic acid and its oxidized form (dehydroascorbic acid) is the most significant chemical property of ascorbic acid [1084] It has been postulated that this vitamin acts as a reducing or oxidizing agent but specific substances affected by it have not been identified satisfactorily [1084] Ascorbic acid (AA) is the reduced or storage form dehydroascoric acid (DHA) is the oxidized or active form These two forms occur in some sort of equilibrium in which the actual amount of AA greatly exceeds that of the DHA form Perhaps this bears some relation to the fact that DHA may be reduced to AA (which is reversible), or DHA may be metabolized to the irreversible non vitamin form diketogulonic acid [1084]

Physiologists have not accepted the function of the oxidized form of ascorbic acid as a hydrogen acceptor in animals as freely as in plants Brobeck[113] states that evidence indicates that ascorbic acid functions in hydrogen transport in plant metabolism but a similar function has not been verified in animals Meiklejohn[676] suggests that the reversibility of the oxidized and reduced form of ascorbic acid seems to assure for it a role in tissue oxidation Bach[3] states that hydrogen transfer in plants is mediated through the glutathione ascorbic acid path However he cites Barron (1939) who points out that most investigators do not consider ascorbic acid as functioning in oxidation reduction systems

The reversibility of the AA and DHA forms of ascorbic acid often depends upon the simultaneous reversibility of glutathione Ascorbic acid and its oxidation products may have a catalytic function in the formation of S—bonds between protein and collagen or may function in the oxidation of organic sulfur to sulfates [61] Bach[3] points out that ascorbic acid is a strong biological reductant and that it may be a reactivator for SH groups as well as glutathione maintaining ascorbic acid in the reduced state Numerous other substances including adrenalin and purines with free amino groups (*e g* xanthine uric acid and theophylline) inhibit the oxidation of vitamin C [346] Some substances such as flavones stabilize ascorbic acid and also catalyze oxidation in low concentration [81] Needham [33] considers that the function of vitamin C in the synthesis of AMP is due to

in scorbutic guinea pigs, and the hemorrhagic syndrome mentioned by Clark[164] in human cases of insufficient vitamin C In addition, wounds in scorbutic men and animals do not have increased phosphatase[108]

Ascorbic acid has a capacity for preventing or minimizing the toxic effects of an imposing number of different conditions, drugs and minerals These include alkaptonuria (tyrosinosis), analgesics, certain anesthetics, antipyretics arsphenamines, barbiturates, barium, Benzedrine (amphetamine), benzene, bismuth sodium thiodiglycollate, gold chloride, certain hepatoxic agents, lead, certain conditions of leukopenia, certain infectious diseases, mercurial diuretics, certain test reactions (e g, tuberculin reaction), influenza virus, "certain strains of poliomyelitis," procaine, sulfonamides (especially sulfapyridine), toxins of several bacteria and trichlorethylene,[78] certain steroids, phenol tetanus toxin[378] and other substances Bicknell and Prescott[78] review the literature on ascorbic acid in detoxications of many substances including benzene Detoxication of benzene also occurs by conversion to phenol, and by conjugation with glucuronic acid[407] It is believed that detoxication by ascorbic acid is not the basis for its antiscorbutic activity[378]

Meiklejohn[676] notes that some of the ailments for which ascorbic acid is used "have nothing in common with scurvy" He believes that the idea that ascorbic acid increases resistance to infection and aids in detoxification processes is founded on dubious clinical and laboratory evidence" Nevertheless, Liethman[603] and others call attention to the fact that carbohydrate and a derivative glucuronic acid, participate in "special chemical reactions which transform certain poisons into relatively innocuous substances

The method by which ascorbic acid functions in detoxication has not been determined Bourne[98] considers that intracellular ascorbic acid may detoxify toxins within the cell and that it may function by attaching to the molecule to make substances water soluble in a manner similar to that proposed for the adrenal gland in which vitamin C makes corticosteroids water soluble Because glucuronic acid has been credited with the ability to detoxicate some of these substances consideration should be given to whether ascorbic acts directly or has an indirect function in the oxidation of glucose to glucuronic acid The relative significance of the direct action of ascorbic acid as compared with its indirect effect in the formation of glucuronic acid, has not been recognized

Ascorbic acid has been considered a determining factor in the virulence of the diptheria toxin Bicknell and Prescott[78] review the controversial literature on this subject They point out that many workers have stated that the degree of virulence of a standardized diptheria toxin, as measured by the guinea pig assay is dependent on the ascorbic acid intake of the

and associates[97] found that healing was not delayed in non infected wounds in neutropenic guinea pigs, however, when infection occurred in control animals polymorphonuclear leukocytes accumulated, but were absent in the neutropenic animals at the site of the wound

Kobak and collaborators [3] found that protein deficiency caused a delay in wound healing in rats from the 3rd through the 5th postoperative day and that cicatrices in these rats had a low tensile strength which was attributed to the failure of fibroblasts to form reticulum and to delay in the transformation of retention collagen into mature collagen Other investigators have found that protein deficiency delayed the rate of wound healing but that it became morphologically normal after the addition of methionine to the deficient diet [9] Some of the cicatrices that sometimes form after an operation for glaucoma [33] may be due in part to protein deficiency

Most conditions which are favorable for the growth of fibroblastic tissue are also favorable for epithelial proliferation However, Olson and co workers[75] point out that urea can decrease the formation of epithelium, and increase the formation of granulation tissue They found that urea retards the formation of epithelium in skin wounds that are healing by granulation but that it stimulates the rate of formation and extension of granulation tissues in rabbits

Some agents that have little effect on wound healing are usually those agents or substances that have little effect in increasing or decreasing lymphoid tissue For example Carrel and co workers[147] found that the rate of healing of wounds was not modified by dressings impregnated with distilled water or with hypertonic sodium chloride solution However, substances such as benzpyrene dissolved in olive oil (1 0 and 0 07 per cent) produce a slight temporary delay in the early stages but this effect was not evident in the last stage of the healing process[933]

Extracts of embryonic tissues stimulate growth in tissue cultures and the healing of wounds[713, 734] Injections of embryonic extracts[735, 1074] extracts of adult tissues[634] nucleotides[441, 3] and allantoin[370, 86] increase wound healing under experimental conditions Needham[735] states that the action of embryonic extracts is due to its growth promoting substance' One growth promoting principle of animal tissue was found to be similar to that in yeast and to contain adenine guanine pentose and phosphorous however it did not contain protein pyridine or sulfur[616] Carrel[144, 145] preferred plasma that contained leukocytes to tissue extracts Morosov and Striganova[717] report that wound healing in rats was more rapid had less inflammation, and had more granulation when treated with human embryo extracts than in the untreated wound of the opposite side which served as a control Wilmer and colleagues found that the effect of embryo extract is so strong that it initiated new growth in tissue cultures after growth had

ascorbic acid transferring oxygen to glutathione, and that oxidized glutathione activates alkaline phosphatase.

Synthesis of glucuronic acid. The effects of a deficiency of ascorbic acid suggest that it has a function in the synthesis of glucuronic acid, but not in the synthesis of glucosamine which is not decreased in scorbutic pigs.[631] Quick[819] states that the two scorbutic guinea pigs used in their experiment synthesized glucuronic acid. Miller, Sichrs and Brazda[696] also found that guinea pigs on a vitamin C free diet were unable to synthesize glucuronic acid, "or that the synthesis proceeds very slowly." Mosbach and King[719] state that scorbutic guinea pigs can synthesize glucuronic acid, but they caution that this may not be indicative of the normal method of synthesis since 'the mechanism of glucuronide formation may differ, depending on the need of the animal *for glucuronic acid for detoxication or synthesis of* tissue uronide.'

Failure of the oxidation of glucose to glucuronic acid may alter the synthesis of ascorbic acid because glucuronic acid, in turn, is a source of vitamin C. Although, as King points out, there is no clear evidence that indicates the actual precursors of ascorbic acid in plants and animals, glucuronic and galacturonic acid are common nutrients which might serve as precursors.[164] Glucose is a source of ascorbic acid, as indicated in several types of experimental studies and radioactive tracer studies have also shown that glucose is the source of ascorbic acid in rats,[4 0] and probably in many mammals except guinea pigs and primates. Jackel[4 0] used radioactive tracers and found that glucose is a source of ascorbic acid in rats. He and his co-workers report that the carbon chain is used directly in this synthesis.[969] Guha and Ghosh (1934, 1935) suggest that other hexoses produce greater increases in the synthesis of ascorbic acid. They state that mannose produced *the greatest increase in the synthesis of ascorbic acid in* plants and animals. Von Sztareczky (1938) also considers mannose a precursor of vitamin C.[164]

PROTEIN IN WOUND HEALING

Most conditions which decrease wound healing have a systemic effect in decreasing the extracellular plasma protein level. Thereby these conditions also inhibit intracellular retention of protein by lymphocytes and plasma cytes. A decreased rate of wound healing in patients and animals that received prolonged administration of cortisone has been well established. X rays, nitrogen mustard, adrenocorticotropic hormone (ACTH), cortisone, infection, suppuration, chronic chemical irritation, foreign bodies and various other lymphopenic agents also delay healing. Although the administration of sulfonamide drugs may produce sulfanemia, Cannaday[139] reports that this condition did not interfere with wound healing. Lawrence

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acid insoluble, or C portion that has a high concentration of deoxyribo nucleic acid (DNA), and an acetone insoluble or E, fraction which is rich in RNA

The formation of plasmacytes from lymphocytes in regions of wound healing is a normal process *in vivo* It contributes to fibroblastic prolifera tion because the abundant cytoplasm of plasma cells is very rich in RNA which is important in protein synthesis A number of works on the trepho cytic relations of lymphocytes in regeneration is reviewed by Needham [734] who concludes that the effective healing substances are nucleoproteins The high nucleoprotein content of thymic material, which is primarily DNA promotes regeneration

Excretory products of protein, particularly urea and allantoin increase wound healing Maggots promote healing of wounds in man [443] Stock raisers in Texas and other southwestern states learned nearly 2 centuries ago that if an indolent wound was infected by "screw worms" for a few days and the maggots exterminated, the wound speedily healed Since the excretion of the maggots contains urea, which is known to promote wound healing it has been suggested that the beneficial effects of maggot infestation on wound healing are due to the destruction of necrotic tissue and the excre tion of allantoin by the maggots [443] Imms[461] found that urea is as effective as allantoin in stimulating healing when it is applied to wounds He states that some non healing wounds in man such as x ray burns and osteomyeli tis have been successfully treated with allantoin and urea

The administration of proteins or their products is another method of stimulating wound healing An increased rate of wound healing following the application of various chemicals has been attributed to hyperemia or increased vascularization The favorable effect of epicutan on the regenera tion of muscle and collagen has been attributed to improved vasculariza tion [3] The rate of healing of experimentally produced wounds has been increased by pre and postoperative treatment with varied agents, such as ultraviolet radiation of rats and dogs [408] porphyrin [1 3] and calcium precipi tating agents [253] Unguentolan a cod liver oil ointment supposedly rich in vitamin A improved wound healing over that obtained by applying a vita min A free control ointment to skin wounds in guinea pigs [0] Members of the sulfhydryl group stimulated regeneration in the hermit crab [400] stimu lated proliferative reaction in the skin of guinea pigs [839] and increased the rate of healing of burns [443] *p* Thiocresol, a proprietary "sulphydryl sub sti tution product of cresol" [38] is effective in promoting epithelial growth if it is applied after granulation has begun [443] The extent to which these sub stances increase capillary dilation and permeability has not been deter mined

ceased.[734] However, Doljanski and Auerbach[238] report that their experiments showed that embryonic extract, applied locally, definitely inhibited wound healing in the skin of rats. Extracts of adult tissues have also been reported to stimulate wound healing. Extract of fowl heart stimulated the growth of human epidermis.[38]

Necrosis and cell injury release growth promoting substances. Loofbourrow and co-workers[616] found that an aqueous extract of minced embryonic chick, newt or rat tissues, which had been previously injured by lethal ultraviolet radiation, contained a growth promoting substance that was 2 to 9 times as potent as that obtained from normal, non-irradiated tissues of these animals when "assayed by quantitative yeast growth methods." The growth factor, thus obtained, retained its potency after being dried or autoclaved. Epstein and Frankal believe that the apparent source of wound hormones is the components released by the disintegration of cells in the wound, and that wound hormones that are obtained from viscera stimulate the growth of skin, but those obtained from skin are incapable of stimulating healing in viscera.[716] The chemical stimulus which causes rapid mitosis of fibroblasts in incisions into fibrous tissue is believed to be liberated from degenerating cells.[100] Carrel believes that removing debris and blood clots from a sterile wound decreased the rate of healing.[100] Clerc[170] found that the presence of red blood cells hastened healing. Sanguinin, a tryptic hydrolyzate of erythrocytes which in preparation is "finally lyophilized to a brown powder," is antibacterial and showed considerable ability to stimulate granulation tissue and to shorten the healing time of experimental wounds in dogs and of abcesses in dogs and cats.[33]

The nature of growth promoting factors has been attributed to peptic digested protein.[734] Carrel and Baker claim that "the ability to promote growth is directly proportional to the ease with which the protein of a tissue can be transformed to peptides by enzymes."[713] Hammett and Reimann, however, believe that the "sulfhydral radical is one of the most essential stimulating agents."[713] Baker and Carrel reported that the growth promoting power of proteoses plus thymonucleic acid was about equal to embryonic extract, and that nucleoproteins from cow embryos, prepared by the method of Hammarsten, had great growth promoting effect. This fraction of embryonic tissues contained both "thymo- and phytonucleoproteins."[353] The fact that nucleoproteins increased growth is especially interesting to us because a lysis of lymphocytes and plasmacytes releases nucleic acids, histones and gamma globulins.

Growth promoting effects have been attributed to nucleic acids. Fischer (1939) stated that the growth promoting effect of embryo extracts on fibroblasts was due to ribose nucleotide.[13] Needham[734] showed that the growth promoting fraction in embryonic extract may be separated into an

occurs in smaller wounds which are not complicated by jagged or anemic necrotizing edges infection loss of tissue or other conditions precluding apposition of the edges of the wound *Healing by granulation tissue was formerly called* secondary healing'[6 5] or healing by second intention[833] More recently, a third intention as been recognized Thus Dorland[30] defines healing by second intention as 'union by the adhesion of granulating surfaces and healing by third intention' as healing by granulation of 'union by the filling of a wound with granulations Mallory[673] points out that the terms primary and secondary are essentially one and the same process and all gradations between the two extremes exist

Storage of nucleoproteins in cells in lymphoid tissues is related to systemic protein anabolism An increase in lymphoid tissue represents an increased intracellular storage of protein Agents which deplete the lymphoid tissues and cause pronounced lymphopenia inhibit or prevent growth and wound healing Agents that increase lymphoid tissue promote a higher level of several plasma proteins and a consequent increase in rate of growth and wound healing In 1916 Zeleney[1133] noted that wound healing was largely controlled by factors which were not inherent in the cells near the cut surface Holmes[443] cites the works of several investigators who recommend high protein high polyvitamin diets to promote repair in burned areas and other serious wounds Others advocate that a high calorie high polyvitamin diet be given to patients for 2 weeks before and following surgery

Inanition

Starvation protein and/or vitamin deficient diets decrease the rate and quality of wound healing Some of the decrease in these systemic conditions may be due to inadequate amounts of ascorbic acid inhibiting collagen formation Other effects however are due to inadequate plasma protein a condition that is commonly concomitant with systemic depletion of lymphoid tissue Protein high diets contribute directly to wound healing through plasma proteins and indirectly by increasing lymphocytes and plasmacytes which concentrate nucleic acids amino acids some minerals and probably certain enzymes which have a part in the processes of mitosis

Prolonged impairment of nucleic acid and protein synthesis by nutritional deficiencies decreases infiltration of lymphocytes and their transformation into plasmacytes as well as the growth of fibroblasts The retarding and generally deleterious effects of starvation on cicatrization of skin wounds were observed as early as 1828 by de Martigny[471] Chudnovsky (1890) studied wound healing of the skin in starved rabbits and reported that the regeneration of epidermal cells was retarded that the

Lymphocytes and Plasmacytes

Agents which increase lymphocytes and plasmacytes increase the rate of cellular growth, whereas those which deplete lymphoid tissues decrease the growth rate These parallel effects are related because small lymphocytes and plasmacytes synthesize and store protein and nucleic acids However, the infiltration of lymphocytes and the formation of plasmacytes should not be considered essential for the mitosis of cells but only as an accessory means by which nucleic acids, histones and other proteins are transported and concentrated

"Round cell infiltration' was a favorite term employed by many investigators to describe the aggregation of a variety of free cells, including lymphocytes and plasmacytes, at the site of wound healing In 1949 this practice of lumping cells was denounced[8] Plasmacytes do not usually occur in blood or lymph except in certain pathological conditions They do not infiltrate a tissue as do lymphocytes Plasmacytes in healing wounds develop from lymphocytes, most of which infiltrated the area

Formation of plasmacytes during wound healing is related to the increase of protein in the tissue fluid The time required for healing and the number of plasmacytes which develop vary with histamine or serotonin release the amount and nature of the necrotic material the vascularity and amount of plasma proteins, the presence or absence of infection, and the situation and extent of the wound[534] Thus the original extent and type of the wound (chemical contused open incised lacerated, thermal etc) and its management (whether sutured, open, plastically treated cystic infected, etc) determine whether the lesion heals by first intention' or by 'granulation

The concentration of hydrogen ions plays a little appreciated part in wound healing A pH around 7 0 appears to be the turning point in human wounds for a pH of less than 7 0 is usually painful, whereas a pH of more than 7 0 favors healing[443] Alkaline phosphatases are more active in the nearer younger cells in healing wounds than in the older more distant cells This is equally true but occurs more rapidly in wounds produced in cultures of chick embryos which really represent an instance of regeneration within a regenerating system'[6 4] Consequently these factors and conditions may play an important part in the extent and duration of exudation the protein content of the exudate the infiltration of lymphocytes and the resulting fibrinogenesis and plasmacytogenesis[534]

Wound healing is similar in most tissues[6] and in animals and men[13] McCutcheon[663] simplifies the approach to grasping the differences of healing with or without the formation of granulation tissue by stating Repair is said to be primary where the edges of the wound are in apposition secondary if the wound gapes Healing without granulation (commonly referred to as primary healing direct union or healing by first intention)

of only one third that of controls on a normal diet on the 5th postoperative day[5,3] However, Kobak and co workers[553] were unable to demonstrate any relationship between various reduced plasma protein or various degrees of moderate weight loss and tensile strength of completely healed wounds It has been stated that the reduced growth rate in hypoproteinemia is due to changes in osmotic pressure which have been relieved by the administration of acacia Koster and Kasman (1942) suggest that wound disruption may be due to a poor nutritional state of which the hypoproteinemia is merely a manifestation and not the cause[84]

Deficiency of essential amino acids or other smaller units has been considered a cause for the decreased rate of wound healing in animals on a low protein diet Formation of scar tissue in albino rats was reported to *be as effective when a small amount of methionine was added to the pro*tein free diet as when the rats were kept on the normal diet[95] However fibroplasia does not occur in the absence of ground substance which is dependent upon a *normal* supply of ascorbic acid[78] and *polysaccharides* some of which may be released from mast cells[5,7,5,8]

Deficiency of many of the fractions of the vitamin B complex depletes lymphoid tissues This lymphoid depletion actually represents the disappearance of the individual s reserve nucleic acid and most of his essential amino acids In addition the interference with nucleic acid and protein synthesis is manifested in decreased growth processes including repair total body growth embryonic development and hematopoiesis each of which is similarly affected by the same vitamin deficiency The effects of the avitaminoses commonly involve the disturbance of so many functions that it is difficult or impossible to determine which vitamin is lacking Zimmerman[1135] illustrates this point by stating So frequently are these avitaminoses associated with anorexia vomiting and diarrhea with their attendant malnutrition that the organism soon shows evidence of a lack of such basic essentials as protein and certain salts

Cortisone

Cortisone depresses the formation of ground substance fibroblasts and blood vessels[8,6] This *steroid is a potent lymphocytopenic agent* and its depletion of lymphoid tissue and production of lymphopenia result in a decrease in the number of plasmacytes Not only does cortisone decrease wound healing by the depletion of plasma protein and lymphocytolysis but also by reducing capillary permeability Banthine and Dibenamine also inhibited vascularization and granulation tissue formation in turpentine abscesses in white rats The fibroblasts intercellular substance and the vascular pattern of the inhibited granulation tissue had the same appearance regardless of which of the suppressing agents was administered

mitotic figures were reduced in number, often abnormal in form and poor in chromatin, that granulation tissue was either absent or diminished, and that there were few or none of the infiltrating cells which were present in the healing wounds of the well nourished control animals[471]

The time sequence of infiltration of lymphocytes and plasmacytes is related to conditions existing in the tissue fluid incident to the transformation of lymphocytes to plasmacytes in wound healing and follows essentially the same pattern as in inflammatory reaction Needham[34] believes that a "flow" period is characterized chiefly "by extensive mobilization of protein and persists throughout the 2nd and 3rd week of healing in serious wounds, or reaches the maximum "from the fourth to the eighth day after lighter wounds" The age of the animal is a factor in inanition, for it has been shown that healing was retarded in young rats on a reduced amount of food, but that repair of gastric wounds was not affected by total inanition in adult rats[451]

The relation of protein deficient diets to a decreased rate of wound healing has been observed clinically Vines[1059] comments that "burns heal slowly because the protein loss has depleted the protein reserves of the body", infection and subsequent suppuration with protein loss further retard healing This statement is supported by the indications that a high protein diet is required by convalescents in whom processes of repair are active[1059] Moise and Smith[70] compared the effect of high fat and high carbohydrate diets upon wound healing in rats, and found that the most rapid rate of healing was in animals on a standard diet and not on the high carbohydrate diet as Whipple and co workers observed in dogs Moise and Smith report however, that the standard diet produced the highest activity in repair, whereas the high fat diet produced the lowest, and, that there was "little if any difference between the carbohydrate and protein diets" This similarly may be due to the fact that animals on a high carbohydrate diet often have a more favorable flora for intestinal synthesis of B vitamins and other factors A deficiency of ground substance precursor must also be considered in evaluating the need for a high protein diet in wound healing

Harvey and Howes show that after repair had been initiated a high protein diet increased the velocity and efficiency of healing with the result that the maximum strength of the healing wound was reached about 2 days earlier than with a standard diet[443, 713] Other investigators report hypoproteinemia as the cause of failure of wound fibroplasia thus resulting in the formation of scar tissue that had such low tensile strength that disruption of surgical wounds resulted in dogs[443] Low protein diet hampered wound healing and caused defective fibroplasia and a delay in healing of approximately 2 days with scars that had a tensile strength

unless the infectious process which may prove to be a preneoplastic or neoplastic growth, is removed [70] Multiple colonic polyposis is believed to be inherited as a simple dominant trait in man [38] Lockhart Mummery (1934) states that the occurrence of polyposis in which the lesions may be numerous and distributed throughout the entire colon or limited to segments of it (adenomatosis) is a familiar disease If this condition is not adequately treated it is practically certain to develop into carcinoma [46] Earlier observers Bickersteth (1890), Cripps (1882), Port (1895) and Smith (1887) preindicated the above conclusions since they thought that intestinal polyposis exhibits an hereditary individual and local predisposition [93]

Ewing [93] tentatively classified all mucosal papillary tumors in four groups Nasal and uterine polyps which result from inflammatory hyperplasia and for this reason fall into the field of productive inflammation are placed in his first group polypoid adenomas of mucous membranes which result from the overgrowth of atypical tissues are placed in his third group He also warns that in addition to the difficulties of applying this classification it may be extremely difficult to determine whether a mucous polyp is purely inflammatory or partly neoplastic Kinschensky suggests that the processes involved in chronic inflammation including later metaplasia accompanied by gradual hyperplasia may result in papillomatous growths in the urinary tract [332] As would be expected lymphocytes plasmacytes eosinophils and some polymorphonuclear leukocytes are seen intermingling with the epithelial elements in the metaplastic epithelium of areas of mucosal papillomas and polyps The pathological picture including the numerous inflammatory cells shows that the soft papillomas of papillary hypertrophic sinusitis are lesions of an inflammatory nature [70, 93] in which drainage of edematous fluid is inhibited [6] However polyps arising from the mucosa of the nares and sinuses vary histologically [27] Rhinoscleroma arises very similarly to nasal polyps and characteristically this growth is densely infiltrated by plasmacytes [7] Many perhaps most of the subchronic and chronic cases of inflammation of mucous membranes are attended by a pronounced increase in plasmacytes Forbus[313] illustrates this point strikingly in cases of chronic cholecystitis and in chronic polypoid nasopharyngitis

Several types of polyps and mucosal papillomas are attributed to inflammatory hypertrophy that inhibits or even obstructs the venous or lymphatic drainage so as to produce stasis This condition usually involves a small delimited area of the mucosa with the result that the protein content of the exudate is appreciably increased over that of normal plasma lymph or intercellular fluid and contains numerous plasmacytes Some writers and investigators [39, 93, 401, 981] believe that such a condition of inflam

except that the ground substance was only quantitatively diminished[1011, 101]

Taubenhaus and co workers[1011, 101] apparently did not suspect that the observed reduction in vascularity caused by the action of the two sympathetic ganglion blocking agents (Banthine and Dibenamine) may have effectively inhibited the exudation of tissue fluid, protein, lymphocytic infiltration, plasmacytogenesis and the formation of ground substance in the area of expected granulation However, in noting the histological changes they state that leukocytes were present in the Dibenamine treated rats, but they do not indicate whether these cells were as numerous as in controls or whether the leukocytes were monocytes, polymorphonuclear leukocytes and/or lymphocytes and plasmacytes They regard, however, most of the changes in the fibroblasts, ground substance and capillaries as being due primarily to decreased capillary function The length of mucopolysaccharide (MPS) chains in the formation of granulation tissue is not altered by cortisone[1011] It is claimed that pituitary growth hormone completely counteracted cortisone induced, local inhibition of granulation tissue and caused ample granulation tissue to form in hypophysectomized rats[101] Other investigators[464] believe that cortisone and ACTH decrease the number of mast cells which in itself, would cause a decrease in the formation of collagenous fibers

Anterior pituitary growth hormone increases nitrogen retention,[1100] and increases the amount of lymphoid tissues Its effect in increasing wound healing however has not been established[1100] Williamson and Numann[1100] explained these results as indicating that large doses of the growth stimulating hormone "mobilize protein metabolites primarily for use by the body tissue to the detriment of the healing wound tissue, small amounts of growth hormone permit greater diversion to the regenerating wound tissue" It is not clear whether or not the growth hormone which they used was ACTH free and there is no explanation for the loss of 16 g body weight of the control rats during the 20 day duration of the experiment

Mucosal Polyps and Papillomas

The occurrence and growth of mucosal polyps and papillomas apparently are dependent upon conditions of partial stasis with impeded drainage of protein rich tissue fluid such as have repeatedly been pointed out in this work as being favorable if not prerequisite for the transformation of infiltrated lymphocytes into plasmacytes Thus plasmacytes are usually abundant in polyps which are believed to represent an edema and simple hypertrophy of the nasal mucosa resulting from an inflammatory process"[70] Soft, often diffused nasal and other mucous papillomas and polyps are variously regarded as an inflammatory hyperplasia that recurs

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Ewing[93] states that inflammatory reaction with the appearance of lymphocytes and plasmacytes is a highly significant feature of malignant tumor growth and must be regarded as a defensive process' He supports this point of view by calling attention to the 'islands of tumor cells enclosed in masses of lymphocytes and presenting clear signs of degeneration' which may be found in carcinoma of the breast and to the fact that 'one finds a thick barrier of lymphocytes about many slow growing epitheliomas As explained in the next section on conditioning hosts the idea that lymphocytes function as a direct defense against malignant tumor growth is untenable

Conditioning Hosts

Lymphoid tissues are changed by several methods of host conditioning in preparation for heterologous transplantation For example cortisone decreases the growth rate Injections of various lymphoid tissues such as spleen or thymus into animals have been made for the purpose of determining whether or not injected lymphocytes altered the immunity or defense reaction Murphy[7 7] found that the Jensen rat sarcoma would grow when implanted even when serially transplanted into 6 to 18 day old chick embryos and into the forebrain of x rayed animals of different species They concluded that the failure of growth of heterotransplants under usual conditions is not due to a lack of suitable nutrient material as Ehrlich s theory of athrepsia maintains These investigators found that the introduction of native lymphoid tissue with the foreign graft successfully counteracted the absence of resistance in the chick embryo and the adult brain to heterotransplants These results and the observation that "accumulation of the small round cells about foreign tissue is a constant finding were chiefly responsible for their discarding the antibody explanation and concluding that lymphocytes are directly responsible for host resistance to heterotransplants

Murphy s[7 7] observations seem to be unimpeachable However Stevenson (1917) using the same type of rat sarcoma and essentially the same technique that Murphy used failed to obtain results supporting Murphy s finding that when chicken spleen was included with the sarcoma transplant the contained lymphocytes stimulated the chorioallantoic membrane to resist the growth of the implanted tumor[894] Sandstrom[894] likewise transplanted normal embryonic renal tissue of ducks to the chorioallantoic membrane of chicken eggs but also failed to obtain any adverse effect on growth by including adult chicken spleen in varying quantities with the transplant However we feel that Murphy erred in ascribing a "militant function to the kindly lymphocyte which functions as a trephocyte in all

matory stasis and hyperplasia of the mucosa stimulates the development of mucous polyps Others point out that lymph stasis is important in the development of spontaneous cancer[293, 401]

An inflammatory origin, in which there is edema and increased protein in the edema fluid with infiltration of lymphocytes and transformation to plasmacytes, has been described for polyps and papillomas that occur in the mucosa of various structures and organs Thus, plasmacytes have been observed at sites which suggest that these cells were transformed from lymphocytes under conditions of inflammatory stasis, and that plasmacytes in the edema fluid supplied some of the RNA to promote the growth of mucosal polyps or papillomas in the bronchi,[981] in the cervix uteri,[4, 633] endometrium,[93, 633, 748] colon,[293, 713] conjunctiva,[1019] lips,[107] larynx,[93] middle ear,[981] nares[?0, 293, 6 5, 1059] nasopharynx,[313] stomach,[93, 713] tonsillar region,[70] umbilical cord,[1017] renal pelvis,[581] ureter[293] and urinary bladder[293, 581]

INFILTRATION IN TUMOR GROWTH

Infiltration of neoplastic growth, especially in the earlier stages, is more variable than is usually the case in wound healing, for the conditions incident to the site of the lesion are somewhat reversed In a simple, incised wound, the injured and dying cells are in close proximity to normal, healthy tissues in an early, well established tumor, local anemia usually causes death of cells in central areas of the growth which are more or less isolated from the normal tissues by a thick, peripheral wall of proliferating tumor tissue Later as areas of central necrosis of the tumor coalesce and spread, varying numbers of lymphocytes plasmacytes, other leukocytes and mast cells probably will be found in edematous areas outside of the growing tumor Although lymphocytes, plasmacytes and mast cells may be abundant in the tissues surrounding the tumor, comparatively few or no mast cells will be found within the necrotic or proliferating tissue of transplanted sarcoma or carcinoma[08]

We[35] have observed the infiltration of lymphocytes in and around spontaneous mammary carcinomas in mice a basaloma and myxosarcoma in hamsters and biopsy of human mammary adenocarcinoma The cause of the variation in amount or extensiveness of the infiltration is obscure for the activities of the lymphocytes and plasmacytes in infiltration of tumors is highly variable within different tumors within a single section of a given tumor, and within the adnexa of the tumor Vascularity, necrosis and edema which affect the growth rate all appear to play variable parts in the infiltration of a tumor The plasmacytic infiltrations are much more readily seen in tissues fixed with sublimate alcohol than in those fixed in formalin, Zenker's, Helley's or Bouin's solutions

tumor implants has generally been overlooked Since plasmacytes are re garded as an important source of antibodies several authors have recently considered the presence of these cells as being significant Favour [95] states it is pre umed that lymphocytes and plasmacytes which infiltrate the site of graft rejections are producing antibodies *in situ* which perhaps do not appear in detectable amounts in the circulation and yet can elicit local anaphylactic responses of the polyarteritis or Arthus phenomenon type Although the plasmacytes in these sites may function in the formation of antibodies they probably have other functions The plasmacytes at the site of degenerating cancer grafts may also have the same function as those found at sites of wound healing and chronic inflammation that is they supply nucleoprotein to form a time bridge over the gap between the period of acute inflammation and the onset of the proliferation of fibro blasts and/or various tissues which form the site of the tumor graft

In the implantation of homo and heterotran plants conditions are pres ent which might evoke two different functions of plasmacytes (1) the formation of antibodies to the foreign protein and (2) provision for RNA and protein synthesis for the purpose of serving as a protein time bridge for the homotransplant such as occurs at sites of chronic inflammation and wound healing

SUBCHRONIC INFLAMMATION AND CARCINOGENESIS

A variety of essentially subchronic inflammatory conditions have been indicted as being potentially procarcinogenic cancer promoting precar cinogenic a causative factor or even the cause of cancer Broussais (1772, 1838) firmly believed that cancer is the sequel of recurrent inflammation and that cancer never arises in normal tissue but only after inflammatory alteration [93] This physiological doctrine of Broussais as an ex planation of the origin of cancer made a great impression at the time and may be considered as the only tenable point in the present day doctrine of irritation as the cause of cancer Ewing [93] states that Billroth s dictum without previous chronic inflammation cancer does not exist while sub ject to exceptions is yet so generally true as to establish the great sig nificance of chronic irritation as a factor in tumor genesis More recently with the general acceptance of Friedewald and Rous (1944) terms ini tiating action and promoting factor [74] it would seem that in the light of the concept of a two stage mechanism subchronic inflammation is more closely related as a promoting agent than as a directly causative process in carcinogenesis Berenblum [4] indicates that the two stage mechanism of carcinogenesis which is ba ed upon the concept of dormant tumor cells and therefore, does not take into consideration tumor viruses other stages of carcinogenesis and an untold number of recognized and unknown fac

growth processes In this case, one is unable definitely to evaluate the results of the experiments because of the following possibilities

1 Adult chicken lymphocytes would probably not have the same effect in the tissues of a chick embryo

2 Introduction of proteins from adults would probably involve the injection of foreign proteins, such as enzymes, antibodies and others, that are not present in the egg

3 Injection of lymphocytes from an adult would also represent the injection of several enzymes, such as deoxyribonuclease (DNAase) and certain proteolytic enzymes, which probably would be present in increased amounts The disintegration of a graft in an adult might be due to the presence of antibodies, whereas the disintegration of a tumor implant in an embryo might be due to proteolytic enzymes

4 Because of the relatively great amount of stroma in chicken spleens, the number of available lymphocytes introduced with the spleen transplant may not have been significant

5 Apparently little is known of the fate of the transplanted lymphocytes, that is, whether they persisted, disintegrated or transformed into plasmacytes Therefore, little significance can be attached to their trephocytic value under these conditions

Effect of Site on Transplanted Tumor

Several sites, such as the cerebrum and anterior chamber of the eye of adult animals and embryos have less lymphocytic reaction than occurs in subcutaneous or other loose connective tissues Siebert observed that homiotransplants of thyroid gland into the brain of guinea pigs survived for a longer period and that there was less lymphocytic reaction involved than around subcutaneous implants [6,14] However Murphy and Sturm[7,8] reported an absence of cellular reaction about a heteroplastic graft in the brain and point out that this absence may be due to a mechanical factor which inhibits the infiltration of lymphocytes beyond the perivascular space, or that the brain is an uncongenial environment for lymphoid cells If the infiltration of lymphocytes is a reaction due to increased plasma proteins, the infiltration may be via the blood lymph or cerebrospinal fluid, and not extend into the tissue fluids within the cerebrum This explanation does not seem to be tenable in view of Murphy's finding that implants into the posterior lateral part of the cerebrum and the cerebellum were not satisfactory for the growth of heteroplastic tissue grafts

The infiltration of lymphocytes around tumor implants has added significance in view of the two widely accepted conclusions that plasmacytes develop from lymphocytes and that plasmacytes are a source of antibodies The significance of plasmacytic aggregation in growing *and in regressing*

tumor implants has generally been overlooked Since plasmacytes are regarded as an important source of antibodies, several authors have recently considered the presence of these cells as being significant Favour [2] states it is presumed that lymphocytes and plasmacytes which infiltrate the site of graft rejections ' are producing antibodies *in situ* which perhaps do not appear in detectable amounts in the circulation and yet can elicit local anaphylactic responses of the polyarteritis or Arthus phenomenon type Although the plasmacytes in these sites may function in the formation of antibodies they probably have other functions The plasmacytes at the site of degenerating cancer grafts may also have the same function as those found at sites of wound healing and chronic inflammation that is they supply nucleoprotein to form a time bridge over the gap between the period of acute inflammation and the onset of the proliferation of fibroblasts and/or various tissues which form the site of the tumor graft

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tors and/or conditions, nevertheless represents "a working hypothesis of tumor pathogenesis"

It appears that the processes involved in the broader aspects of sub chronic inflammation, especially in areas of loose connective tissue, could supply the essential, initiating, promoting and/or synergising substances or factors for provoking the alteration of normal, dormant tumor em bryonic or other cells by chemical, physical and/or other processes so as to incite them into neoplastic growth or to render them susceptible to cancer inciting virus or other stimuli This, in turn, may result in the un controlled growth of tissues and the supply of the necessary substrates and enzymic systems to promote frank carcinogenesis

A wide range of subchronic inflamamtory conditions have been thought by various writers, to contribute to, or actually initiate, neoplastic growth Some of the many cases in which this view has been expressed are chronic mastitis, indolent or incompletely extirpated ulcer , mucous polyps, be nign tumors, fistulous tracts and cracked, especially fissured, leukoplakia of the tongue, [93] hyperplasias of the breast, prostate gland, or uterine mucosa[59] and many other subchronic or chronic, but probably not re current[663] or acute conditions Some authors have gone so far as to believe that it is necessary to control such external factors as long continued ir ritation of any sort remove all pigmented moles which are exposed to irritation "[613] adequately treat all 'foci of chronic irritation" and safe guard the individual from contact with occupational carcinogens [41]

Levin[599] credits Virchow as probably being one of the earliest investi gators to believe "that some external agent in the presence of a nutritive or formative and a functional stimulus might 'incite a cell to prolifera tion' "[534] Deelman (1922) noted during the first 80 days (the first of his three stages) of tar carcinogenesis that the epidermal cells underwent an orderly type of hypertrophy and hyperplasia with an increase in the 'elas tic tissue of the hyperemeic corium' accompanied by the infiltration of lymphocytes and mast cells [93] Notwithstanding these significant observa tions, apparently neither Deelman nor Ewing associated the obvious pres ence of mast cells with the need for increased *capillary permeability or production of an anticoagulant or the invasion of lymphocytes* as in any way supporting Virchow s hypothesis

Formerly, it was generally believed that hyperemia is the most essential fact of inflammation and is caused by paralysis of the vasomotor nerves (paralytic theory) [39] Although this is probably applicable to spontaneous (*neurogenic*) vasodilation and has been described in hypertrophic mou e skin following applications of tar [379 380 381] as far as we are aware little *attention* has been paid to the problem of whether or not histamine, leuko taxin,[68] serotonin[884] or other substances which dilate and increase the

permeability of blood capillaries operate by locally affecting vasomotor neurons during localized stasis Kotzareff (1926) and Redmond and as o ciates (1925) found that excision of the superior cervical sympathetic ganglion on the same side as the ear which was repeatedly tarred markedly shortened the latent period compared with the time required for unoperated rabbits [73]

Berenblum[73] succeeded in producing malignant tumors in a small per centage of the mice repeatedly subjected to "mild freezing of the skin with Dry Ice over a long period ' but, when both Dry Ice and tar were used, the incidence and degree of malignancy, as well as the latent period of the tumors more closely approached these conditions in the tumors pro duced by tar alone

Continued percutaneous applications of tar to mice or rabbits result in the proliferation of lymphatic capillaries[814] and 'the occurrence of ex cessively dilated capillaries" with paralleling hyperemia of the epithelium These conditions accompanied by a certain amount of stasis and an in creased transudation, supply the hyperemic tissue 'with supernormal amounts of nourishing substances and oxygen, which is followed by hyper trophy of the epithelium formation of warts papillomas and finally, the development of malignant carcinomas [564] Other investigators[190 19 304 3 9 111 1123 11 4] report finding these changes in the skin of mice and rabbits after repeated application of tar 3 4 benzpyrene (which is the most widely distributed substance in carcinogenic tars)[111] or other polycyclic hydro carbon carcinogens commonly used in experimentally producing cutaneous cancers We[5 0 535] obtained similar sequences by percutaneous applications of 9,10 dimethyl 1 2 benzanthracene to hamsters which developed hy pertrophy of the corium papillomas melanomas and, later, epitheliomas

Some investigtaors state that tissue changes are first manifested by the epidermis after percutaneous application of tars or their carcinogenic de rivatives This change occurs in the first week of painting ' in mice and rabbits [373] Others state that the connective tissue cells in the corium are the first affected and that the epithelial changes are secondary [379] Krey-berg[564] believes that vascular changes are first effected and that the re sultant hyperemia may be due to changes in the blood vessels them elves or to a disturbance in their innervation—or that the circulatory disurb ances may have their origin in the connective tissue ground substances or stroma Results of Guldberg s[379] experiments confirm the findings of earlier authors by showing that the hyperaemia is the first demonstrable change occurring in the skin during painting and it is attended by increa ed tran sudation edema and cellular migration He[3 9] points out that these series of changes are members of the symptom complex' characteri tic of in flammation due to an irritant and concludes that the e findings favor

"the view that there must exist an intimate relationship between the changes in the vessels and the epithelial changes" which show progressive hyperplasia of follicular epithelium, followed by carcinogenesis Levy and associates[601] found that a single application of a very strong carcinogen (0 6, 2 0 or 4 0 per cent 9,10 dimethyl 1,2 benzanthracene in benzene) to the lip of mice was followed by "hyperplasia of epithelial cells and islands of epithelium in the dermis" and by the presence of "numerous inflammatory cells in the subepithelial connective tissue " (The mucous membrane was refractory to the carcinogen)

Berenblum [4] states that "all the known promoting agents are irritant He defines irritation as "unphysiologic stimulation which, being potentially destructive, elicits a continued state of reparative hyperplasia Knight[5 0] expressed the opinion that all tumors are not due to chronic irritation, for "intermittent irritation or pressure with intermittent attempts a repair, produces and keeps present the necessary type of capillary for rapid growth of any cells " The popular "irritation hypothesis ' of the origin of neoplasia is tenable only in terms of subchronic inflammatory processes in which there is present repeatedly alternated injurious (inflammatory) and reparative proce ses that may eventually result in uncontrolled proliferation and neoplastic growth Klein[543] found that if the ears of young adult mice were painted a single time with 0 5 per cent methylcholanthrene in olive oil and beginning 2 weeks later they were painted consecutively at least 30 times with croton oil tumors would be promoted by the croton oil, which is believed to cause hyperemia but not carcinogenesis

The idea of repetitive attempts at healing being related to carcinogenesis is further supported by the usual results of percutaneous application of chemical carcinogens Repeated applications of the carcinogen or a single *application of the carcinogen plus multiple applications of a non carcinogenic promoting agent* [74] produce neoplasia If the applications in some instances are discontinued after several months and after papillomas have appeared as shown by Rous and Kidd (1941) in rabbits, the healing processes *may suppress the no longer abetted carcinogenic* proces es with the result that the papillomas disappear and the lesions heal [749] Klein[543] states that Salaman (1952) failed to obtain skin tumors after painting mice only once with 9 10 dimethyl 1 2 benzanthracene followed by a single painting with croton oil Klein obtained few or no vi ible tumors after painting the skin of mice a single time with methylcholanthrene followed by one application of croton oil He explains the effects of repeated applications of croton oil following the carcinogen as demonstrating that promotion un like initiation i a gradual process This tatement leads one to wonder just which one of the multiple applications of the chemical carcinogen

which are usually necessary to produce cancer, should be credited with having 'initiated' carcinogenesis

The irritation hypothesis in itself is untenable as an explanation of the cause of cancer It is obvious that multiple factors and conditions including a latent period probably having a duration of 5 months to 20 to 30 years are involved Thus in order to become tumorigenically effective, irritation would be expected to play the part of subchronic inflammation in which there are overlapping repetitions of increased capillary permeability with leakage of protein accompanied by more or less edema Cowdry and Paletta[193] found that the viscosity of the nucleoplasm of epidermal cells, rendered hyperplastic by percutaneous treatment with methylcholanthrene was lower than in normal epidermal cells in mice They found in man that the viscosity of the nucleoplasm of the hyperplastic epithelial cells at the base of warts and at the site of healing wounds was lower, and that the nucleoplasmic viscosity in cells of squamous carcinomas was much lower than in normal cells These are noteworthy observations because it is well known that changes in viscosity alter membrane permeability and that viscosity changes are indicative of changes within the cell or its medium

The origin of neoplastic growth is thought to be related to a disturbance of the normally balanced or "steady state" of proliferative cells with regard to their chemicophysiological relations to the intercellular and circulating fluids Although the maintenance of this steady state may be dependent upon a number of factors, it has been postulated that the enzyme systems are probably the most sensitive and, therefore the most vulnerable to factors that might disturb the normal state of balance in proliferating cells This little understood steady state has been regarded as a delicate trigger mechanism depicted as a cofactor, a coenzyme and an enzyme that forms the sides of an equilateral triangle Any defection of one of these members of the enzymic system would distort or pull the 'trigger' which in one out of many instances may provoke a series of changes that eventually may result in frank neoplasia[193] Whether the results of the series of changes occurring in carcinogenesis should be regarded as mutational or chemicophysiological is not clear Nevertheless most investigators recognize a latent period of several weeks to 5 to 30 years between the initiation of the cause and the appearance of cancer

Literature Cited

1 ACKERMAN L V *Surgical Pathology* C V Mosby Company St Louis 1953

2 AGRAWALA I P DUNCAN C W AND HUFFMAN C F J Nutrit 49 29 1953 (Biol Abst 27 #21633)

3 ALBRITTON E C (ed) *Standard Values in Blood AF Tech Rept No 6039* Wright Patterson Air Force Base Dayton Ohio 1952

4 ALLFREY V G MIRSKY A E AND OSAWA S J Gen Physiol 40 451 1957

5 ALTSCHUL R *Endothelium Its Development Morphology Function and Pathology* The Macmillan Company New York 1954

6 ALTSCHULER C H AND ANGEVINE D M In *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 178 Ejnar Munksgaards Forlag Copenhagen 1954

7 ANDERSON W A D (ed) *Pathology* p 956 C V Mosby Company St Louis 1953

8 DE ANDINO A M JR RIVERO FONTAN J L AND PASCHKIS K E Proc Soc Exper Biol & Med 77 700 1951 (Biol Abst 26 #14887)

9 ANDREASEN E Acta path et microbiol scandinav Suppl 49 1 1943

10 ANDREW W AND ANDREW N V Anat Rec 93 251 1945

11 ANDREW W AND SOSA J M Anat Rec 97 63 1947

12 ANFINSEN C B *Fat Metabolism* edited by V A Najjar p 93 Johns Hopkins Press Baltimore 1954

13 ANGELAKOS E T J Pharmacol & Exper Therap 119 444 1957

14 ANGEVINE D M *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 46 Acta Inc Montreal 1953

15 ANTOPOL W GLAUBACH S AND GRAFF S Proc Soc Exper Biol & Med 86: 364 1954

16 AREY, L B *Human Histology* W B Saunders Company Philadelphia 1957

17 ARTZ N E AND OSMAN E M *Biochemistry of Glucuronic Acid* Academic Press Inc New York 1950

18 ASBOE HANSEN G Acta dermat venereol 30 338 1950

19 ASBOE HANSEN G Acta dermat venereol 30 27 1950

20 ASBOE HANSEN G Ann Rheumatic Dis 9: 149 1950

21 ASBOE HANSEN G Acta dermat venereol 30 221 1950

22 ASBOE HANSEN G Proc Soc Exper Biol & Med 80 677 1952

23 ASBOE HANSEN G Cancer Res 13 587 1953

24 ASBOE HANSEN G in *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 274 Ejnar Munksgaards Forlag Copenhagen 1954

25 ASBOE HANSEN G LEVI H AND WEGELIUS O Cancer Res 17 79? 1957

26 ASCHOFF L Pp 515–641 in *Pathologische Anatomie* Vol ? p 545 Gustav Fischer Verlag Jena 1911

27 ASH J E Pp 698–732 in *Pathology* edited by W A D Anderson p 698 C V Mosby Company St Louis 1953

28 ASHTON N AND COOK C Brit J Exper Path 33: 415 195?

29 ATKINSON W B AND LEATHEM J H Anat Rec 95 147 1946

30 AUSTIN C R Exper Cell Res 4 249 1953

31 *BABES A J Physiol et Path Gen* 28 56? 1930 (Biol Abst 6 #10165)

32 BACH S J *The Metabolism of Protein Constituents in the Mammalian Body* Oxford University Press London 195?

33 BADER M N Texas Rep Biol & Med 10: 773 1952 (Biol Abst 28 #11115)

34 BAECKELAND E AND LECOMTE J

Compt rend Soc biol **147** 927 1953
35 Bagliomi T Tumori **38** 267 1952 (Biol Abst 28 #8317)
36 Baier W E Ann New York Acad Sc **61** 639 1955
37 Bailey F R and Miller A M *Text Book of Embryology* Ed 2 William Wood & Company New York 1911
38 Baker B L Ann New York Acad Sc **56** 684 1953
39 Banfield W G Anat Rec **114** 157 1952
40 Barki V H Derse P H Collins R A Hart E B and Elvehjem C A J Nutrition **37** 443 1949 (Biol Abst 24 #5930)
41 Barnard W G Cancer Rev April 1,7 1931
42 Barnes J M Brit J Exper Path **21** 264 1940
43 Barnett R J in *Histology* edited by R O Greep p 273 Blakiston Company New York 1954
44 Barrnett R J and Seligman A M J Nat Cancer Inst **14(4)** 769 1954
45 Barron D H *A Textbook of Physiology* edited by J F Fulton p 733 W B Saunders Company Philadelphia 1949
46 Bartlett P W and Peck M E Surg Gynec & Obst **90** 547 1950
47 Baserga A de Nicola P and Vahi P Experientia **6** 471 1950
48 Bass A D and McArdle H Fed Proc **13** 334 1954
49 Bassett C Andrew L., and Meyer K Anat Rec **124** 481 1956 (#TC 9)
50 Bassiouni M J Clin Path (British) **6** 39 1953 (Am J Clin Path Abst **24** 87)
51 Bates E O Anat Rec **61** 231 1935
52 Battaglia B Pubbl d stazione zool di Napoli **22** 79 1949 (Biol Abst 24 #14650)
53 Bayed E D Blood **3** 987 1948
54 Beach E F., Bernstein S S., Hummel F C Williams H H and Macy I G J Biol Chem **130** 115 1939
55 Beams H W *Biological Symposia* Vol X p 71 Jaques Cattell Press Lancaster Pa 1943
56 Beams H W and King R L Biol Bull **71** 188 1936
57 Beattie J M and Dickson W E C *A Textbook of Pathology General and Special* Ed 5 Vols I and II William Heinemann Ltd London 1948
58 Begg R W Proc Am A Cancer Res **1(1)** 5 1953
59 Bell E T *A Textbook of Pathology* Ed 6 Lea & Febiger Philadelphia 1947
60 Bell G H Davidson J N and Scarborough H *Textbook of Physiology and Biochemistry* Ed 2 The Williams & Wilkins Company Baltimore 1953
61 Benditt E P Fed Proc **15** 507 1956
62 Benditt E P., Bader S Arase M Corley C and Lam K B Fed Proc **13** 422 1954
63 Benditt E P Bader S and Lam K B A M A Arch Path **60** 104 1955
64 Benditt E P Humphreys E M Straube R L., Wissler R W and Steffee C H J Nutrition **33** 85 1947
65 Benditt E P Wong R L Arase M and Roeper E Proc Soc Exper Biol & Med., **90** 303 1955
66 Benhamou E and Nouchy A Compt rend Soc biol., **109** 401 1932 (Biol Abst 7 #22189)
67 Bennati D., and Patetta A Arch Soc biol Montevideo **14** 73 1947
68 Bennett G A *Pathology* Ed 2 Edited by W A D Anderson p 1248 C V Mosby Company St Louis 1953
69 Bensley R R *Special Cytology* edited by E V Cowdry p 199 Paul B Hoeber Inc., New York 1932
70 Bensley R R., and Vimtrup B Anat Rec., **39** 37 1928

71 BENSLEY S H Anat Rec 60 93 1934

72 BENTLEY O G JOHNSON R R HERSHBERGER T V CLINE J H AND MOXON A L J Nutrition 57 389 1955

73 BERENBLUM I Brit J Exper Path 10 179 1929

74 BERENBLUM I *Advances in Cancer Research* edited by J P Greenstein and A Haddow p 129 Academic Press Inc New York 1954

75 BESSIS M *Cytology of the Blood and Blood Forming Organs* translated with additions by Eric Ponder Grune & Stratton Inc New York 1956

76 BEST C H AND MCHENRY E W Physiol Rev 11 371 1931

77 BEST C H AND TAYLOR N B *The Physiological Basis of Medical Practice* Ed 3 The Williams & Wilkins Company Baltimore 1943

78 BICKNELL F AND PRESCOTT F *The Vitamins in Medicine* Grune & Stratton Inc New York 1953

79 BIGELOW F S J Lab & Clin Med 43 759 1954

80 BING J F Acta med scandinav 103 547 1940

81 BIRGER E K Fysiograf Sallskapet Lund Forhandl 17 155 1947 (Biol Abst 25 #4192)

82 BISSET K A *The Cytology and Life History of Bacteria* The Williams & Wilkins Company Baltimore 1950

83 BJØNEBOE H AND GORMSEN H Acta path et microbiol Scandinav 22 649 1943 (Biol Abst 19 #8795)

84 BLACKFAN K D DIAMOND L K AND LEISTER C M *Atlas of the Blood in Children* Commonwealth Fund New York 1944

85 BLAND J H *Clinical Recognition and Management of Disturbances of Body Fluids* Ed 2 W B Saunders Company Philadelphia 1956

86 BLASCHKO H *Histamine* edited by G E W Wolstenholme and C M O'Connor p 381 Little Brown & Company Boston 1956

87 BLAXTER K L J Comp Path & Therap 60 127 1950 (Biol Abst 25 #33900)

88 BLOOM G FRIBERG U LARSON B AND ABERG B Acta path et microbiol scandinav 37 163 1955 (Biol Abst 30 #13761)

89 BLOOM W *Radiation Biology* edited by A Hollaender Vol I Part 2 p 1091 McGraw Hill Book Company Inc New York 1954

90 BODANSKY M *Biochemistry of Disease* Ed 2 The Macmillan Company New York 1952

91 BOHM A A VON DAVIDOFF M AND HUBER G C *A Textbook of Histology* Ed 2 translated W B Saunders Company Philadelphia 1901

92 BOYNES G J Ann New York Acad Sc 61 721 1955

93 BORGES F J AND BESSMAN S P Proc Soc Exper Biol & Med 93: 513 1956

94 BORSOOK H J Cell & Comp Physiol 47 (Suppl 1) 35 1956

95 BORST M *Pathologische Anatomie* edited by L Aschoff Vol I 2nd impr p 555 Gustav Fischer Verlag Jena 1911

96 BOURNE G H *An Introduction to Functional Histology* Little Brown & Company Boston 1953

97 BOURNE G H *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder Vol II p 42 Academic Press Inc New York

98 BOURNE G H *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder Vol II p 1 Academic Press Inc New York 1953

99 BOWES J H ELLIOTT R G AND MOSS J A *Nature and Structure of Collagen* edited by J T Randall p 199 Academic Press Inc New York 1953

100 BOYD W *A Text Book of Pathology* Ed 4 Lea & Febiger Philadelphia 1945

101 BOYD W C *Fundamental of Im*

munology Ed 2 Interscience Publisher New York 1947

102 Bozović Lj and Milković S Experientia 10 8 1954

103 Brachet J *Embryologie Chimique* Masson et Cie Paris 1944

104 Brachet J *Chemical Embryology* translated by L G Barth Interscience Publishers New York 1950

105 Brachet J *Nucleic Acid (Symposium Soc Exper Biol No 1)* p 207 University Press Cambridge England 1951

106 Brachet J *Le role des acides nucleiques dans la vie de la cellule et de l'embryon* Masson et Cie Paris 1952

107 Brachet J *The Nucleic Acids Chemistry and Biology* edited by E Chargaff and J N Davidson Vol II p 519 Academic Press Inc New York 1955

108 Bradfield J R G Biol Rev Cambridge Philos Soc 25 113 1950

109 Briggs R and King T J *Biological Specificity and Growth* edited by E G Butler p 20 Princeton University Press Princeton 1955

110 Brilla G Arch ges Physiol 257 287 1953

111 Bristol L D J A M A 72 1048 1919

112 Brobeck J R *A Textbook of Physiology* Ed 16 edited by J F Fulton p 1047 W B Saunders Company Philadelphia 1949

113 Brobeck J R *A Textbook of Physiology* Ed 16 edited by J F Fulton p 1069 W B Saunders Company Philadelphia 1949

114 Brodie B B and Shore P A *Hormones Brain Function and Behavior* edited by H Hoagland p 161 Academic Press Inc New York 1957

115 Brody G Science 118 770 1953

116 Brown N C The effects of various B vitamin deficiencies on the pouch and associated glands in the golden hamster (*Cricetus auratus*) Thesis University of Colorado Department of Biology 1946

117 Brownson R H Anat Rec 118 284 1954 (#108)

118 Bruck C Acta Dermat Venereol 30 125 1950

119 Bruda B E Ztschr Krebsforsch 34 185 1931 (Biol Abst 7 #5897)

120 Bruton O C Med Ann District of Columbia 22 618 1953 (Biol Abst 28 #18183)

121 Bueno P Science 106 347 1947

122 Bunting C H *Special Cytology* edited by E V Cowdry p 683 Paul B Hoeber Inc New York 1932

123 Burgi E Deutsche med Wchnschr 68 893 1942 (Biol Abst 17 #20451)

124 Burnet F M and Fenner F *The Production of Antibodies* Ed 2 Macmillan Co Ltd London 1949

125 Burstone M S Anat Rec 115 543 1953

126 Burton A C Physiol Rev 34 619 1954

127 Busacca G Arch ital otol 39 582 1928 (Biol Abst 5 #18512)

128 Butler L O and Morgan A F Proc Soc Exper Biol & Med 85 441 1954

129 Butterworth C E Jr Frommeyer W B Jr and Riser W H Jr Blood 8 519 1953 (Biol Abst 27 #31960)

130 Bywaters E G L and Glynn L E *Biochemical Disorders in Human Disease* edited by R H S Thompson and E J King p 673 Academic Press Inc New York 1957

131 Calhoun M L *Microscopic Anatomy of the Digestive System of the Chicken* Iowa State College Press Ames 1954

132 Callender S T and O'Brien J R P *Biochemical Disorders in Human Disease* edited by R H S Thompson and E J King p 81 Academic Press Inc New York 1957

133 Cambel P Conroy C E and Scouris J T Science 115(2988) 3 3 1952

134 Cameron A J *Systemic Ophthalmology* edited by A Sorsby p 62 C V Mosby Company St Louis 1951

135 Cameron G R *Pathology of the Cell*

Charles C Thomas Springfield Ill 1951
136 Cameron G R *The Suprarenal Cortex* edited by J M Yoffey p 155 Academic Press Inc New York 1953
137 Campbell B and Good R A Ann Allergy 7 741 1949
138 Campbell R M Innes I R and Kosterlitz H W J Endocrinol 9 52 1953 (Biol Abst 28 #10791)
139 Cannaday J E Ann Surg 119 498 1944 (Biol Abst 18 #16900)
140 Cannon P R *Advances in Protein Chemistry* edited by M L Anson and J T Edsall Vol II p 135 Academic Press Inc New York 1945
141 Cappell D F *Muir's Text Book of Pathology* Ed 6 The Williams & Wilkins Company Baltimore 1951
142 Carelton H M and Leach E H *Schafer's Essentials of Histology* Ed 15 Lea & Febiger Philadelphia 1949
143 Carlson J G *Radiation Biology* edited by A Hollaender Vol I Part 2 p 763 McGraw Hill Book Company Inc New York 1954
144 Carrel A J Exper Med 36 385 1922
145 Carrel A J A M A 82 255 1924
146 Carrel A and Ebeling A H J Exper Med 28 513 1923
147 Carrel A du Nouy and Carrel A J Exper Med 26 279 1917
148 Caspersson T O *Cell Growth and Cell Function A Cytochemical Study* W W Norton & Co Inc New York 1950
149 Cathcart E P J Physiol 32 299 1905
150 Cavallero C *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 87 Acta Inc Montreal 1953
151 Cavanagh J B *An Outline of the Cytology and Pathology of the Reticular Tissue* edited by A H E Marshall p 214 Oliver & Boyd Ltd Edinburgh 1956
152 Cerletti A *Neuropharmacology* edited by H A Abramson p 9 Josiah Macy Jr Foundation New York 1956
153 Chambers R *General Cytology* edited by E V Cowdry p 237 University of Chicago Press Chicago 1924
154 Chantel E Arch Ist biochem ital 2 457 1930 (Biol Abst 5 #27486)
155 Charkey L W Wilgus H S Patton A R and Gassner F X Proc Soc Exper Biol & Med 73 21 1950
156 Cheng E W and Thomas B H Proc Iowa Acad Sc 59 178 1952 (Biol Abst 27 #21674)
157 Chessin M Dubnick B Kramer E R and Scott C C Fed Proc 15 #1334 1956
158 Chevremont M and Firket H *International Review of Cytology* edited by G H Bourne and J F Danielli Vol II p 261 Academic Press Inc New York 1953
159 Chiodi H Endocrinology 26 107 1940
160 Christensen H N *Amino Acid Metabolism* edited by W D McElroy and B Glass p 63 Johns Hopkins Press Baltimore 1955
161 Christman A A Physiol Rev 32 303 1952
162 Churney L Biol Bull 82 52 1942
163 Clark E R and Clark E L Am J Anat 35 265 1925
164 Clark G W *A Vitamin Digest* Charles C Thomas Springfield Ill 1953
165 Clark I and Umbreit W W Proc Soc Exper Biol & Med 86 558 1954 (Biol Abst 22 #8012)
166 Clark W S Ropes M W and Bauer W *Proceedings First Clinical ACTH Conference* edited by J R Mote p 337 Blakiston Co Philadelphia 1950
167 Claude A *Biological Symposia* Vol 10 p 111 Jaques Cattell Press Lancaster Pa 1943

168 Clausen H J Proc Soc Exper Biol & Med 83 835 1953
169 Clawson B J and Nerenberg S T J Lab & Clin Med 42 746 1953
170 Clerc W O Bull d'hist appliq a la physiol 15 277 1938 (Biol Abst 13 #5673)
171 Clopton J R Personal communication 1956
172 Coassin N A and Kline D L Am J Physiol 190 147 1957
173 Cochrane R L and Meyer R K Proc Soc Exper Biol & Med 96 155 1957
174 Code C F *Blood Cells and Plasma Proteins Their State in Nature* edited by J L Tullis p 292 Academic Press Inc New York 1953
175 Code C F *Histamine* edited by G E W Wolstenholme and C M O Connor p 189 Little Brown & Company 1956
176 Coggeshall H C *Comroe s Arthritis and Allied Conditions* Ed 5 edited by J L Hollaender p 48 Lea & Febiger Philadelphia 1953
177 Cohen P P *Chemical Pathways of Metabolism* edited by D M Greenberg p 1 Academic Press Inc New York 1954
178 Cole L J and Ellis M E Cancer Res 14 738 1954
179 Cole L J Fishler M C Ellis M E and Bond V P Proc Soc Exper Biol & Med 80 112 1952
180 Combes T J C Rev Soc argent biol 9 186 1933 (Biol Abst 9 #9229)
181 Compton A S Am J Anat 91 01 1952
182 Conklin E G *General Cytology* edited by E V Cowdry p 539 University of Chicago Press Chicago 1924
183 Conway E A Anat Rec 69 487 1937
184 Conway E J *The Biochemistry of Gastric Acid Secretion* Charles C Thomas Springfield Ill 1953
185 Cooke W E British Med J 1 433 1930
186 Coons A H Leduc E H and Connolly J M Ann New York Acad Sc 69 951 1955
187 Cooper Z K *Cowdry s Problems of Ageing* Ed 3 edited by A I Lansing The Williams & Wilkins Company Baltimore 1952
188 Corner G W *Special Cytology* edited by E V Cowdry p 1567 Paul B Hoeber Inc New York 1932
189 Cowdry E V *General Cytology* edited by E V Cowdry p 313 University of Chicago Press Chicago 1924
190 Cowdry E V J A M A 135 408 1947
191 Cowdry E V *Laboratory Technique in Biology and Medicine* Ed 2 The Williams & Wilkins Company Baltimore 1948
192 Cowdry E V *Advances in Cancer Research* edited by J P Greenstein and A Haddow p 57 Academic Press Inc New York 1953
193 Cowdry E V and Paletta F X Am J Path 17 335 1941
194 Crabb E D Biol Bull 53 67 1927
195 Crabb E D Arch Entwicklungsmechanik Organismen 124 332 1931 (Abst D Ztschr wiss Biol)
196 Crabb E D Stain Technol 24 87 1949
197 Crabb E D *Principles of Functional Anatomy of the Rabbit* Ed 2 Privately published Boulder Colorado 1951
198 Crabb E D Terminology Section Transplant Bull 1(3) 124 1954
199 Crabb E D X Ray News 26 3 1954
200 Crabb E D Univ Colorado Studies Ser in Biol No 4 1 1958
200a Crabb E D Univ Colorado Studies Ser in Biol No 4 36 1958
201 Crabb E D and Kelsall M A J Colorado Wyoming Acad Sc 2(5) 38 1939
202 Crabb E D and Kelsall M A J Morphol 67 351 1940
203 Crabb E D and Kelsall M A J

Colorado-Wyoming Acad Sc 3 44 1940
204 Crabb E D and Kelsall M A Anat Rec 81(Suppl. 4) 64 1941 (#81)
205 Crabb E D and Kelsall M A J Colorado Wyoming Acad Sc 3(2) 59 1942
206 Crabb E D and Kelsall M A J Nat Cancer Inst 12 91 1951
207 Crabb E D and Kelsall M A Proc Am A Cancer Res 1 10 1953
208 Crabb E D and Kelsall M A Proc Am A Cancer Res 2 101 1956
209 Crabb E D and Kelsall M A Radiation Res 5 608 1956
210 Crabb E D and Kelsall M A Anat Rec 124 395 1956 (#300)
211 Crabb E D and Kelsall M A Proc Am A Cancer Res 2(3) 194 1957
212 Crabb E D and Kelsall M A Anat Rec 128 534 1957 (#152)
213 Cramer W J Nat Cancer Inst 2 379 1942
214 Cramer W Drew A H and Mottram J C Lancet 201 1202 1921
215 Cramer W Drew A H and Mottram J C Lancet 200 963 1921
216 Cramer W and Simpson W L Cancer Res 4 601–615 1944
217 Crampton C F Stein W H and Moore S J Biol Chem 225 363 1957 (Biol Abst 31 #30421)
218 Curtiss C Endocrinology 45 284 1949
219 Cuthbertson D P and Phillipson A T *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder Vol II p 128 Academic Press Inc New York 1953
220 Dahlgren U and Kepner W A *A Text Book of the Principles of Animal Histology* The Macmillan Co New York 1908
221 Dalton A J *International Review of Cytology* edited by G H Bourne and J F Danielli Vol 2 p 403 Academic Press Inc New York 1953
222 Dalton A J Kahler H and Lloyd B J Anat Rec 111 67 1951
223 Dann L Glucksmann A and Tansley K Brit J Exper Path 22 70 1941
224 Davidson J N *Symposia of the Society for Experimental Biology No 1 Nucleic Acid* p 77 University Press Cambridge England 1951
225 Davidson J N *The Biochemistry of the Nucleic Acids* John Wiley & Sons Inc New York 1953
226 Dawson A B and Masur J Anat Rec 44 143 1929
227 Deane H W *Histology* edited by R O Greep p 540 Blakiston Company New York 1954
228 Deanesly R Quart J Micros Sc 72 217 1928
229 Dearing W H Jr J Morphol 56 157 1934
230 De Bruyn P P H and Tornova Shehlik M M Anat Rec 119 275 1954
231 Dempsey E W *Histology* edited by R O Greep p 739 Blakiston Company New York 1954
232 Dempsey E W Bunting H and Wislocki G B Am J Anat 81 309 1947
233 Dietrich A *Pathologische Anatomie* edited by L Aschoff Vol I 2nd Impr p 397 Gustav Fischer Verlag Jena 1911
234 Dietrich L S Nichol C A Monson W J and Elvehjem C A J Biol Chem 181 915 1949
235 Diggs L W Sturm D and Bell A *The Morphology of Human Blood Cells* W B Saunders Company Philadelphia 1956
236 Di Stefano H Bass A Diermeier H and Tepperman J Endocrinology 51 386 1952
237 Dohlman C H and Balazs E A Arch Biochem 57: 415 1955 (Biol Abst 30 #19289)
238 Doljanski L and Auerbach F

Proc Soc Exper Biol & Med 55 112 1944

239 DORLAND W A N *The American Illustrated Medical Dictionary* Ed 22 W B Saunders Company Philadelphia 1951

240 DOUGHERTY T F *The Mechanism of Inflammation (International Symposium)* edited by G Jasmin and A Robert p 217 Acta Inc Montreal 1953

241 DOUGLASS C D FELLAS V M MESCHAN I AND DAY P L Fed Proc 13 201 1954 (#668)

242 DOUNCE A L *The Enzymes* edited by J B Sumner and K Myrback Vol I Part 1 p 187 Academic Press Inc New York 1950

243 DOWNEY H Folia haemat I (Teil 11) 275 1911

244 DOWNEY H *George R Minot Symposium on Hematology* edited by W Dameshek and F H L Taylor p 768 Grune & Stratton Inc New York 1949

245 DOWNS A W Blood 3 948 1948

246 DOYLE W L Am J Anat 87 79 1950

247 DOYLE W L *International Review of Cytology* edited by G H Bourne and J F Danielli Vol II p 249 Academic Press Inc New York 1953

248 DRASTICH L Spisy Lekarske Fak Masarykovy Univ Brno Československá Rep 5 193 1927 (Biol Abst 3 #5814)

249 DRENNAN J M J Path & Bact 63 513 1951

250 VON DRIGALSKI W Ztschr Vitamin Hormon u Fermentforsch 3 200 1954 (Biol Abst 10 #15297)

251 DRINKER C K AND YOFFEY J M *Lymphatics Lymph and Lymphoid Tissue Their Physiological and Clinical Significance* Harvard University Press Cambridge 1941

252 DUFRENOY J Biodynamica 4 131 1943 (Biol Abst 18 #9794)

253 DUGAL L P AND LAUGLIER H Rev Canadienne Biol 1 687 1942 (Biol Abst 17 #18842)

254 DUKE ELDER S *Text Book of Ophthalmology* Vol V, *The Ocular Adnexa* C V Mosby Company St Louis 1952

255 DUNGLISON R *A Dictionary of Medical Science* Henry C Lea Philadelphia 1874

256 DURAN REYNALS F *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 103 Ejnar Munksgaards Forlag Copenhagen 1954

257 DURAN REYNALS F AND MCCREA J F *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 160 Acta Inc Montreal 1953

258 DUSTIN A P Compt rend Soc biol belge 90 371 1924

259 DUSTIN A P Compt rend Soc biol belge 93 467 1925

260 DUSTIN A P Bull Assoc Franç etude cancer 14(8) 1 1925

261 DUSTIN A P Compt rend Soc biol belge 93 465 1925

262 DUSTIN A P Ann et Bull Soc Roy Sc Med et Nat Bruxelles No 3-4 1931

263 DUSTIN A P Congres du Cancer de Madrid October 1933 Le Cancer 11(1) 1934

264 DUSTIN A P, AND GREGOIRE C Bull Acad Roy Med Belg p 585 1933

265 DUTRA F R AND MCKIBBIN J M J Lab & Clin Med 30 301 1945 (Biol Abst 20 #15077)

266 ESKERT D AND TOTTERMAN G Scandinav J Clin & Lab Invest 2: 58 1950

267 EDDY W H AND DALLDORFF G *The Avitaminoses* Ed 2 The Williams & Wilkins Company Baltimore 1941

268 EDEN A Nature 145 36 1940

269 EDMUNSON R S AND HALL D A Biochem J 58 34 1954

270 EGGSTON A A AND WOLFF D *Histopathology of the Ear Nose and*

Throat The Williams & Wilkins Company Baltimore 1947

271 EHRICH W E *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 25 Acta Inc Montreal 1953

272 EHRICH W E *Blood Cells and Plasma Proteins* edited by J L Tullis p 187 Academic Press Inc New York 1953

273 EHRICH W E DRABKIN D L AND FORMAN C J Exper Med **90**: 157 1949

274 EHRICH W E, HARRIS T N AND MERTENS E Fed Proc **5** 220 1946

275 EHRICH W E AND SEIFTER J *The Effect of ACTH and Cortisone upon Infection and Resistance* edited by G Schwartzman p 25 Columbia University Press New York 1953

276 EICHENBERGER M Exper Cell Res **4** 275 1954

277 EISENSTARK A AND DRAGSDORF R D Poultry Sc **32** 840 1953 (Biol Abst 28 #6222)

278 ELFTMAN H Anat Rec **118** 147 1954

279 ELKINTON J R AND DANOWSKI T S *The Body Fluids* The Williams & Wilkins Company Baltimore 1955

280 ELMAN R J A M A **128** 659 1945

281 ELWYN D Canad J M Sc **31** 236 1953

282 EMERSON G A Proc Soc Exper Biol & Med **70** 392 1949

283 EMMEL M W J Am Vet M A **97** 145 1940 (Biol Abst 15 #8473)

284 EMMEL, V M Anat Rec **95** 159 1946

285 EMMELIN N KAHLSON G AND WICHSELL F Acta physiol scandinav **2(Pt 2)** 110 1941

286 EMMELIN N KAHLSON G AND WICKSELL F Acta physiol scandinav **2 (Pt 2)** 123 1941

287 ENGLERT M E Stud Inst Divi Thomae (Cincinnati) 4 59 1945 (Biol Abst 20 #15055)

288 ERF L Am J Med Sc **200** 11 1940

289 ERSPAMER V Ricerca Sc **22**: 1568 1953 (Biol Abst 27 #12937)

290 ERSPAMER V, AND ASERO B Nature **169** 800 1952

291 EVANS H M MOON H D SIMPSON M E AND LYONS W R Proc Soc Exper Biol & Med **38**: 419 1938

292 EWING J *Clinical Pathology of the Blood* Lea Brothers & Company Philadelphia 1901

293 EWING J *Neoplastic Diseases A Treatise on Tumors* Ed 3 W B Saunders Company Philadelphia 1931

294 FARR R S Experiments on the fate of the lymphocyte Anat Rec **109** 515 1951

295 FAVOUR C B Transplantation Bull **1** 145 1954

296 FAWCETT D W *Histology* edited by R O Greep p 13? Blakiston Company New York 1954

297 FAWCETT D W Anat Rec **118** 297 1954 (#89)

298 FAWCETT D W J Exper Med **100** 217 1954

299 FAWCETT D W *Histology* edited by R O Greep p 83 Blakiston Company New York 1954

300 FEKETE E *Biology of the Laboratory Mouse* edited by G D Snell p 89 Blakiston Company Philadelphia 1941

301 FELDBERG W *Histamine* edited by G E W Wolstenholme and C M O Connor p 4 Little Brown & Company Boston 1956

302 FENTON P F AND COWGILL G R *A Textbook of Physiology* Ed 16 edited by J F Fulton p 917 W B Saunders Company Philadelphia 1949

303 FERGUSON J H *A Textbook of Physiology Ed 16 edited by* J F Fulton p 540 W B Saunders Company Philadelphia 1949

304 FIBIGER J AND BANG F Det Kgl Dan ke Videnskab Selskab Biol Meddelelser **3**: 1 1921

305 FICHTELIUS K E Acta Anat **19** (Suppl): 1 1953

306 Field E J J Anat **90** 428 1956 (Biol Abst 31 #28522)

307 Fieschi A Arch Sci Biol **31** 341 1929 (Biol Abst 6 #8323)

308 Fink M A Proc Soc Exper Biol & Med **92** 673 1956

309 Finkelstein M and Thomas P A Cancer Res **11** 801 1951

310 Fisher E R and Hazard J B Am J Clin Path **24** 32 1954 (#40)

311 Florey H W and Carleton H M Proc Roy Soc London Ser B **100** 25 1926

312 Forbus W D *Reaction to Injury* Vol I The Williams & Wilkins Company Baltimore 1943

313 Forbus W D *Reaction to Injury* Vol II The Williams & Wilkins Company Baltimore 1952

314 Forkner C E J Exper Med **49** 323 1929

315 Fradkin W Z *The Diagnosis of Diarrheal Diseases* Grune & Stratton Inc New York 1947

316 Frank J A Kumagai L F and Dougherty T F Endocrinology **52** 655 1953

317 Frazer A C *Annual Review of Biochemistry* edited by J M Luck Vol 21 p 245 Annual Reviews Stanford California 1952

318 Freeman L Marx L and Marx W Tissue heparin and mast cells in rats and rabbits Fed Proc **15** 255 1956

319 Freiberg U and Ringertz N R Exper Cell Res **6** 527 1954

320 Freyberg R H *Comroe's Arthritis and Allied Conditions* Ed 5 edited by J L Hollander p 288 Lea & Febiger Philadelphia 1953

321 Freyburger W A Graham B E Rapport M M Seay P H Govier W M Swoap O F and Vander Brook M J J Pharmacol & Exper Therap **105** 80 1952 (Biol Abst 26 #34673)

322 Frey Wyssling A *Submicroscopic Morphology of Protoplasm* Ed 2 (English) Elsevier Publishing Company New York 1953

323 Friedman M and Byers S Proc Soc Exper Biol & Med **90** 496 1955

324 Fritz J C Wharton F D Jr and Henley R M Fed Proc **13** 458 1954 (#1500)

325 Fukuchi K Biol Abst 12 #12801 1937

326 Fullerton H W and Dugoid H L D Blood **4** 269 1949

327 Furman R H Norcia L N Fryer A W and Wamack B S J Lab & Clin Med **47** 750 1956

328 Gaddum J H J Physiol **119** 363 1953 (Biol Abst 27 #29959)

329 Gaddum J H *Histamine* edited by G E W Wolstenholme and C O Connor p 36 Little Brown & Company Boston 1956

330 Gaddum J H *Histamine* edited by G E W Wolstenholme and C M O Connor p 285 Little Brown & Company Boston 1956

331 Gaddum J H Hebb C O Silver A and Swan A B Quart J Exper Physiol **38** 256 1953 (Biol Abst 28 #18008)

332 de Gaetani G Tumori **16** 334 1930 (Biol Abst 8 #1143)

333 Gale E F *A Symposium on Amino Acid Metabolism* edited by W D McElroy and H B Glass p 171 Johns Hopkins Press Baltimore 1955

334 Galloway J F Lancet **221** 1235 1931

335 de Garis C F *Diseases of the Digestive System* edited by S A Portis Lea & Febiger Philadelphia 1953

336 Gatenby J B and Painter T S *The Microtomist's Vade Mecum* Ed 10 Blakiston Company Philadelphia 1946

337 Gates R Bot Rev **8** 337 1942

338 Gay H Anat Rec **120** 728 1954 (#64)

339 Germuth F G Jr and Ottinger B Proc Soc Exper Biol & Med **74** 815 1950 (Biol Abst 25 #2023)

340 Gertner S B Paasonen M K and Gairman N J Fed Proc **16** 299 1957 (#1281)

341 GESLER R M AND MATSUBA M J Pharmacol & Exper Therap **114** 151 1955

342 GIARMAN N H Fed Proc **15** 428 1956 (#1393)

343 GIBSON J G *Blood Cells and Plasma Proteins Their State in Nature* edited by J L Tullis p 197 Academic Press Inc New York 1953

344 GILLMAN J AND GILLMAN T *Perspectives in Human Malnutrition* Grune & Stratton Inc New York 1951

345 GILMOUR J R J Path & Bact **45** 507 1937

346 GIRI K V Sc & Cult **8** 159 1942 (Biol Abst **17** #18233)

347 GLICK D *Techniques of Histo and Cytochemistry* Interscience Publishers Inc New York 1949

348 GLICK D *International Review of Cytology* edited by G H Bourne and J F Danielli Vol II p 447 Academic Press Inc New York

349 GODARD H PALIOS C AND CODOUNIS A J Physiol et Path Gen **30** 640 1932 (Biol Abst 8 #8360)

350 GOLDSMITH G A *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder Vol II p 505 Academic Press Inc New York 1953

351 GOMEZ F T TURNER C W AND REECE R P Proc Soc Exper Biol & Med **36** 286 1937

352 GOMORI G J Histochem **1** 469 1953

353 GONZALEZ G I Boll Inst Estud Med y Biol **8** 79 1950 (Biol Abst 27 #15989)

354 GONZALEZ G I Boll Inst Estud Med y Biol **8** 133 1950 (Biol Abst 2 #15990)

355 GOOD R A J Lab & Clin Med **46** 167 1955

356 GOOD T A GOOD R A KELLEY V C AND GLICK D Am J Physiol **166** 555 1951

357 GOODSIR J AND GOODSIR H D S *Anatomical and Pathological Observations* Simpkin Marshal & Company London 1845

358 GOORMAGHTIGH N *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 37 Acta Inc Montreal 1953

359 GORDON A S Ann New York Acad Sc **59** 907 1955

360 GORDON A S AND KLEINBERG W Proc Soc Exper Biol & Med **38** 360 1938

361 GORER P A Ann New York Acad Sc **39** 365 1955

362 GOTTESMAN J M AND JAFFE H L J Exper Med **43** 403 1926

363 GOULD B S Arch Biochem **4** 175 1944

364 GOULD G M *Gould's Medical Dictionary* edited by J E Scott Blakiston Son & Company Philadelphia 1928

365 GRADWOHL R B H *Clinical Laboratory Methods and Diagnosis* Ed 2 C V Mosby Company St Louis 1938

366 GRAHAM H T LOWRY O H WAHL N AND PRIEBAT M K J Exper Med **102** 307 1955

367 GRANT J *Hackh's Chemical Dictionary* Ed 3 Blakiston Company Philadelphia 1946

368 GRANT W C AND ROOT W S Physiol Rev **32** 449 1952

369 GRECO G AND MARINO C Riv Ist sieroterap ital **27**: 292 1952

370 GREENBAUM F R J Am Pharm A (Scient Ed) **31** 263 1942 (Biol Abst 17 #4914)

371 GREGORY R A *Histamine* edited by G E W Wolstenholme and C M O'Connor p 27 Little Brown & Company Boston 1956

372 GRENELL R C *A Textbook of Physiology* Ed 16 edited by J F Fulton p 214 W B Saunders Company Philadelphia 1949

373 GROB D J Gen Physiol **29** 219 1946 (Biol Abst 20 #11973)

374 GROSSBERG A I AND GARCIA AROCHA H Science **120(3123)**: 762 1954

375 GROSSFELD H Proc Soc Exper Biol & Med **86**: 81 1954

376 GROSSFIELD H MEYER K AND GOD

man G C Anat Rec 124 489 1956 (*TC 22)
377 Guerrant N B and Dutcher R A J Biol Chem 110 233 1935
378 Guggenheim K Leibowitz L and Rakover D Biochem J 55 388 1953 (Biol Abst 28 #13054)
379 Guldberg G Acta path et microbiol scandinav Suppl 8 223 1931
380 Guldberg G *Aerten in the Malig nant Tar Tumors* Tipografica Ma drid-bla s S A 1933
381 Guldberg G Bull Assoc franc etude cancer 23(2) 1934
382 Gustavson K H *The Chemistry and Reactivity of Collagen* Academic Press Inc New York 1956
383 Haddow A Am J Cancer 22 308 1934
384 Hadfield G *Recent Advances in Pathology* Ed 6 edited by G Hadfield p 238 Blakiston Company New York 1953
385 Haenel U *Proceedings Third International Society of Hematology* edited by C V Moore p 331 Grune & Stratton Inc New York 1951
386 Hanning C G *Histamine* edited by G E W Wolstenholme and C M O Connor p 160 Little Brown & Company Boston 1956
387 Hall E M *Pathology* Ed 2 edited by W A D Anderson p 511 C V Mosby Company St Louis 1953
388 Hallanger L E Laakso J W and Schultze M O J Biol Chem 202 83 1953
389 Halliburton W D and McDowall R J S *Handbook of Physiology* Ed 18 P Blakiston s Son & Company Philadelphia 1929
390 Halpern B N *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 228 Acta Inc Montreal 1953
391 Halpern B N *Histamine* edited by G E W Wolstenholme and C M O Connor p 92 Little Brown & Company Boston 1956
392 Ham A W *Special Cytology* edited by E V Cowdry p 980 Paul B Hoeber Inc New York 1932
393 Ham A W *Histology* Ed 2 J B Lippincott Company Philadelphia 1953
394 Ham A W, and Elliott H C Am J Path 14 323 1938
395 Hamilton H L and Koning A L Anat Rec, 117 522 1953 (*7)
396 Hamilton W F *A Textbook of Physiology* edited by J F Fulton p 756 W B Saunders Company Philadelphia 1949
397 Hamilton W F *A Textbook of Physiology* edited by J F Fulton p 750 W B Saunders Company Philadelphia 1949
398 Hammar J A Endocrinology 5 731 1921
399 Hammett F S, and Coward M Biodynamica No 10 1 1936
400 Hammett F S and Smith D W Protoplasma 13 261 1931
401 Handley W S Canad M A J 21 491 1929 (Biol Abst 5 #16181)
402 Harkness R D Marko A M Muir H M and Neuberger A *Nature and Structure of Collagen* edited by J T Randall p 208 Academic Press Inc New York 1953
403 Harland M Anat Rec 77 247 1940
404 Harma R and Suomalainen P Acta physiol Scandinav, 24 90 1951
405 Harris H *Biochemical Disorders in Human Disease* edited by R H S Thompson and E J King p 578 Academic Press Inc New York 1957
406 Harris T N and Harris S J Exper Med 90 169 1949
407 Harrow B *Textbook of Biochemistry* Ed 5 W B Saunders Company Philadelphia 1950
408 Hart D and Sanger P W Arch Surg 38 797 1939 (Biol Abst 14 #2134)
409 Hartwell, J L *Survey of Compounds Which have been Tested for Carcinogenic Activity* Ed 2 Public Health Service Publication No 149 1951 Superintendent of Documents US Government Printing Office Washington 25 D C

410 HAUROWITZ F *Chemistry and Biology of Proteins* Academic Press Inc New York 1950
411 HAUROWITZ F AND CRAMPTON C F Exper Cell Res Suppl 2 45 1952
412 HAWK P B OSER B L AND SUMMERSON W H *Practical Physiological Chemistry* Ed 12 Blakiston Company Philadelphia 1947
413 HAWKINS D F AND ROSA, L M *Histamine* edited by G E W Wolstenholme and C M O Connor p 180 Little Brown & Company Boston 1956
414 HEDBOM A AND SNELLMAN O Exper Cell Res 9 148 1955
415 HEDIN S G Ztschr physiol Chem 125 289 1922
416 HEILBRUNN L V *An Outline of General Physiology*, Ed 3 W B Saunders Company Philadelphia 1952
417 HEILBRUNN L V WILSON W L AND HARDING D J Nat Cancer Inst 11: 1287 1951
418 HEIDELBERGER C *Advances in Cancer Research* edited by J P Greenstein and A Haddow Vol I p 273 Academic Press Inc New York 1953
419 HEILMAN F R AND KENDALL E C Endocrinology 34 416 1944
420 HEILMEYER L AND BEGEMANN H *Atlas der Klinischen Hamatologie und Cytologie* Springer Verlag Berlin 1955
421 HELLSTEN H Ztschr mikrok anat Forsch 13: 43 1928 (Biol Abst 5 #2727)
422 HERBUT P A *Gynecological and Obstetrical Pathology* Lea & Febiger Philadelphia 1953
423 HERNDON J F AND HOVE E L J Nutrition 57: 261 1955
424 VON HERRATH E Ztschr Zellforsch mikrok anat 23: 375 1935 (Biol Abst 10 #9455)
425 VON HERRATH E Verhandl anat gesellsch 85 196 1938 (Biol Abst 12 #3729)
426 HERRIOTT R M J Gen Physiol 34 761 1951
427 HESS A J Comp Neurol 98 69 1953 (Biol Abst 27 #22295)
428 HESS W C LEE C AND NEIDIG B A J Dental Res 31 791 1952 (Biol Abst 27 #16410)
429 HILKS S P AND OPIE E L Proteolytic digestion of red and white blood corpuscles in the spleen Am J Path 18 333 1942 (Biol Abst 16 #16340)
430 HIGGINBOTHAM R D., DOUGHERTY T F., AND JEE W S S Proc Soc Exper Biol & Med 92 256 1956
431 HIGGINS G M Blood 4 1142 1949
432 HIGH E G AND WILSON S S J Nutrition 50 203 1953 (Biol Abst 28 #8031)
433 HIMES M AND MORIBER L Stain Technol 31 67 1956
434 HIMWICH H E *Brain Metabolism and Cerebral Disorders* The Williams & Wilkins Company Baltimore 1951
435 HIS W Ztschr wiss Zool 11 164 and 11 86 1861
436 HISAW F I Proc Soc Exper Biol & Med 23 661 1926
437 HITCHCOCK C H AND EHRICH W Arch Path 9 625 1930 (Biol Abst 8 #750)
438 HITCHCOCK D I *A Textbook of Physiology* Ed 16 edited by J Fulton p 521 W B Saunders Company Philadelphia 1949
439 HOFFMAN F L *Cancer and Diet* The Williams & Wilkins Company Baltimore 1937
440 HOLBROOK W P, HILL D F STEPHENS A L JR KENT L J AND BORDEN A *Proceedings First Clinical ACTH Conference* edited by J R Mote p 386 Blakiston Company Philadelphia 1950
441 HOLLAENDER A GREENSTEIN J P AND JENRETTE W V J Nat Cancer Inst., 2 23 1941
442 HOLLETT C., AND MENG H C Fed Proc 14: 75 1955 (#241)
443 HOLMES A D New England J Med 227: 909 1942
444 HOLT L M JR *Fat Metabolism* ed

ited by V A Najjar p 53 Johns Hopkins Press Baltimore 1954

445 HOLZAPFEL R A Quart Rev Biol 12 65 1937

446 HORN C A Proc Pennsylvania Acad Sc 9 26 1935 (Biol Abst 10 #20757)

447 HORNING E S AND MILLER I D Australian J Exper Biol & Med Sc, 7 151 1930 (Biol Abst 5 #12479)

448 HORSTADIUS S Acta Zool (Stockholm) 9 1 1928

449 HORSTADIUS S LORCH I J AND DANIELLI J F Exper Cell Res 4 263 1954

450 HOWDEN R Gray's Anatomy Descriptive and Applied Lea & Febiger Philadelphia 1913

451 HOWES E L Connective Tissue in Health and Disease edited by G Asboe Hansen p 159 Ejnar Munksgaards Forlag Copenhagen 1954

452 HUANG T C ULRICH H E AND McCAY C M Fed Proc, 13 462 1954 (#1514)

453 HUDACK S S BLUNT J W JR HIGBEE P AND KEARIN G M Proc Soc Exper Biol & Med 72 526 1949

454 HUECK W Verhandl deutsch Gesellsch inn Med 40 (Kongress) 472 1928 (Biol Abst 4 #14521)

455 HUGHES W H Brit J Exper Path, 29 173 1948

456 HUMMEL K P Am J Anat 57 351 1935

457 HUNT T E AND HUNT E A Anat Rec 124 509 1956 (#229)

458 HUTTRER C P Experentia 5 53 1949

459 HWANG J M S LIPPINCOTT S W AND KRUMBHAAR E B Am J Path 14 809 1938 (Biol Abst 13 #2429)

460 IMAGAWA G Folia Endocrinol Japonica 29 111 1953 (Biol Abst 28 #8393)

461 IMMS A D Lancet 237(6058) 668 1939 (Biol Abst 13 #17256)

462 INGLE D J Proc Soc Exper Biol & Med 38 443 1938

463 INGLE D J Proc Soc Exper Biol & Med 44 174 1940

464 INGLE D J AND BAKER B L *Physiological and Therapeutic Effects of Corticotropin (ACTH) and Cortisone* Charles C Thomas Publisher Springfield Ill 1953

465 INTROZZI P Boll Soc Med Chir Pavia 1 1133 1926 (Biol Abst 1 #9171)

466 ISAACS R *Handbook of Hematology* edited by H Downey Vol I p 5 Paul B Hoeber Inc New York 1938

467 ISTOMANOWA T, MJASSNIKOW A AND SWIATSKAJA A Ztschr ges exper Med 52 160 1926 (Biol Abst 2 #14537)

468 ITO T AND KUBOTA K Cytologia 13 337 1944 (Biol Abst 21 #5131)

469 IVERSEN K *Connective Tissue in Health and Disease* edited by G Asboe Hansen Ejnar Munksgaards Forlag Copenhagen 1954

470 JACKEL S S J Biol Chem 187 569 1950

471 JACKSON C M *The Effects of Inanition and Malnutrition upon Growth and Structure* P Blakiston's Son & Company Philadelphia 1925

472 JACKSON D S *Nature and Structure of Collagen* edited by J T Randall p 177 Academic Press Inc New York 1953

473 JACKSON S F *Nature and Structure of Collagen* edited by J T Randall p 140 Academic Press Inc New York 1953

474 JACKSON S F AND RANDALL J T *Nature and Structure of Collagen* edited by J T Randall p 181 Academic Press Inc New York 1953

475 JACOBSON L F AND NOER R J Anat Rec 114 85 1952

476 JACOBSON W *Nature and Structure of Collagen* edited by J T Randall and S F Jackson p 6 Academic Press Inc New York 1953

477 JAGER E Ztschr Wiss Biol 8 5 8 1929 (Abst B Ztschr Zellforsch u

410 Haurowitz F *Chemistry and Biology of Proteins* Academic Press Inc New York 1950

411 Haurowitz F and Crampton C F Exper Cell Res Suppl 2 45 1952

412 Hawk P B Oser B L and Summerson W H *Practical Physiological Chemistry* Ed 12 Blakiston Company Philadelphia 1947

413 Hawkins D F, and Rosa L M *Histamine* edited by G E W Wolstenholme and C M O Connor p 180 Little Brown & Company Boston 1956

414 Hedbom A and Snellman O Exper Cell Res 9 148 1955

415 Hedin S G Ztschr physiol Chem 125 289 1922

416 Heilbrunn L V *An Outline of General Physiology* Ed 3 W B Saunders Company Philadelphia 1952

417 Heilbrunn L V Wilson W L and Harding D J Nat Cancer Inst 11 1287 1951

418 Heidelberger C *Advances in Cancer Research* edited by J P Greenstein and A Haddow Vol I p 273 Academic Press Inc New York 1953

419 Heilman F R and Kendall E C Endocrinology 34 416 1944

420 Heilmeyer L and Begemann H *Atlas der Klinischen Hamatologie und Cytologie* Springer Verlag Berlin 1955

421 Hellsten H Ztschr mikrosk anat Forsch 13 43 1928 (Biol Abst 5 #2722)

422 Herbut P A *Gynecological and Obstetrical Pathology* Lea & Febiger Philadelphia 1953

423 Herndon J F and Hove E L J Nutrition 57 261 1955

424 von Herrath E Ztschr Zellforsch mikrosk anat 23 375 1935 (Biol Abst 10 #9455)

425 von Herrath F Verhandl anat gesellsch 85 196 1938 (Biol Abst 12 #3729)

426 Herriott R M J Gen Physiol 34 761 1951

427 Hess A J Comp Neurol 98 69 1953 (Biol Abst 27 #2?29?)

428 Hess W C, Lee C and Nedig B A J Dental Res 31: 791 1952 (Biol Abst 27 #16419)

429 Hicks S P and Opie E L Proteolytic digestion of red and white blood corpuscles in the spleen Am J Path, 18 333 1942 (Biol Abst 16 #16340)

430 Higginbotham R D Dougherty T F, and Jee W S S Proc Soc Exper Biol & Med 92 256 1956

431 Higgins G M Blood 4 1142 1949

432 High E G and Wilson S S J Nutrition 50 203 1953 (Biol Abst 28 #8034)

433 Himes M, and Moriber L Stain Technol 31 67 1956

434 Himwich H E *Brain Metabolism and Cerebral Disorders* The Williams & Wilkins Company Baltimore 1951

435 His W Ztschr wiss Zool 11 164 and 11 86 1861

436 Hisaw F L Proc Soc Exper Biol & Med 23 661 1926

437 Hitchcock C H and Ehrich W Arch Path 9: 625 1930 (Biol Abst 8 #750)

438 Hitchcock D I *A Textbook of Physiology* Ed 16 edited by J Fulton p 521 W B Saunders Company Philadelphia 1949

439 Hoffman F L *Cancer and Diet* The Williams & Wilkins Company Baltimore 1937

440 Holbrook W P, Hill D F Stephens A L Jr Kent I J and Borden A *Proceedings First Clinical ACTH Conference* edited by J R Mote p 386 Blakiston Company Philadelphia 1950

441 Hollaender A Greenstein J P and Jenrette W V J Nat Cancer Inst 2 23 1941

442 Hollett C and Meng H C Fed Proc 14 75 1955 (#241)

443 Holmes A D New England J Med 227 909 1942

444 Holt L M Jr *Fat Metabolism* ed

516 Kelsall M A Anat Rec 96 391 1946
517 Kelsall M A Proc Soc Exper Biol & Med 61: 423 1946
518 Kelsall M A J Nat Cancer Inst 10 625 1949
519 Kelsall M A Anat Rec 105 213 1949
520 Kelsall M A Acta Unio internat contra cancrum 7 776 1952
521 Kelsall M A Iotan of Iota Sigma Pi 13 11 1953
522 Kelsall M A Univ Colorado Stud Ser in Biol No 4 62 1958
523 Kelsall M A Univ Colorado Stud Ser in Biol No 4 98 1958
524 Kelsall M A and Crabb E D Am J Clin Path 17 925 1947
525 Kelsall M A and Crabb E D Anat Rec 111 499 1951 (#126)
526 Kelsall M A and Crabb E D Science 115 123 1952
527 Kelsall M A and Crabb E D Anat Rec 115 331 1953 (#214)
528 Kelsall M A and Crabb E D Anat Rec 118 393 1954 (#251)
529 Kelsall M A and Crabb E D Anat Rec 125: 600 1956 (#92)
530 Kelsall M A and Crabb E D Anat Rec 124 415 1956 (#338)
531 Kelsall M A and Crabb E D Proc Am Cancer Res A 2 121 1956
532 Kelsall M A and Crabb E D Anat Rec 128 534 1957 (#152)
533 Kelsall M A and Crabb E D Anat Rec 127 426 1957 (#347)
534 Kelsall M A and Crabb E D Ann New York Acad Sc 72 293 1958
535 Kelsall M A and Crabb E D Unpublished data
536 Kendall J I *Microscopic Anatomy of Vertebrates* Ed 3 Lea & Febiger Philadelphia 1947
537 Kent P W and Whitehouse M W *Biochemistry of the Amino Sugars* Academic Press Inc New York 1955
538 Keuenhof W Frankfurt Ztschr Path 46 416 1934 (Biol Abst 10 #1009)
539 Keuning F J and Van der Slikke L J Lab & Clin Med 36 167 1950
540 Kimball R F Hearon J Z and Gaither N Radiation Res 3 435 1955
541 Kind L S Proc Soc Exper Biol & Med 95 200 1957
542 Kirschbaum A Shapiro J R and Mixer H W Proc Soc Exper Biol & Med 72 632 1949
543 Klein M Cancer Res 13 427 1953
544 Klein W Thannhauser S J and Rossi A Hoppe Seyler's Zeitschr Physiol Chem 231 96 1935 (Biol Abst 10 #439)
545 Kleiner I S *Human Biochemistry* Ed 3 C V Mosby Company St Louis 1951
546 Kleinmann H and Stern K G Biochem Ztschr 222 31 1930 (Biol Abst 9 #2571)
547 Klemola E Acta med scandinav 144 119 1952
548 Klemperer P *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 251 Ejnar Munksgaards Forlag Copenhagen 1954
549 Klouwin H M and Michorst J C A *Progress in Radiobiology* edited by J S Mitchell B E Holmes and C L Smith p 514 Oliver & Boyd Ltd Edinburgh 1955
550 Knight B L Am J Med 37 425 1932
551 Knisely M H Anat Rec 65 23 1936
552 Knisely M H Anat Rec 65 131 1936
553 Kobak M W Benditt E P Wissler R W and Steffee C H Surg Gynec & Obst 85 751 1947
554 Koksal M Nature 172 733 1953
555 Kon S K and Porter J W G Biochem & Physiol Nutrition 1 291 1953
556 Koneff A A Endocrinology 35 77 1944
557 Koster A J Kaulbersz J W and Freeman S Am J Physiol 138: 236 1943 (Biol Abst 17 #13254)
558 Kracke R R *Diseases of the Blood*

mikrosk anat) (Biol Abst 5 #9181)
478 James G W III and Abbott L D Jr Metabolism 1 2?9 1952
479 James J and McDonald J R Arch Path 45 6?? 1948
480 Jancsó N and Jancsó Gábor A Experientia 8 465 1952
481 Jancsó N and Jancsó-Gábor A Schweiz Ztschr allg Path 17 589 1954 (Biol Abst 29 #26433)
482 Janold T and Wilter R W J Clin Invest 28: 286 1949
483 Jaques R Bein H J and Meir R Helvet physiol et pharmacol Acta 14: 269 1956 (Biol Abst 31 #17648)
484 Jasmin G and Robert A (eds) The Mechanism of Inflammation Acta Inc Montreal 1953
485 Jeanloz R Science 111 289 1950
486 Jernstrom P Am J Clin Path 24 957 1954
487 Jim R T S and Steinkamp R C J Lab & Clin Med 47 540 1956
488 Johnston P M and Wentz E M J Morphol 91 79 1952
489 Jolly J Compt rend Soc biol 91 351 1924
490 Jolly J and Saragea T Compt rend Soc biol Paris 90 618 1924
491 Jones O P Blood 3 967 1948
492 Jordan H E Handbook of Hematology edited by H Downey p 703 Paul B Hoeber Inc New York 1938
493 Jordan H E A Textbook of Histology Ed 9 Appleton Century Crofts Inc New York 1952
494 Jordan H E Anat Rec 119 325 1954
495 Jordan H E and Kindred J E A Textbook of Embryology D Appleton & Company New York 1926
496 Jordan H E and Looper J B Am J Anat 39 437 1927
497 Jordan H E and Morton C B Am J Anat 61 407 1937
498 Jordan H E and Speidel C C J Exper Med 38 5?9 1923
499 Jordan H E and Speidel C C Anat Rec 26 223 1923
500 Jorpes J E Heparin Its Chemistry Physiology and Applications in Medicine Oxford University Press Oxford 1939
501 Jukes T H B Vitamins For Blood Formation Charles C Thomas Publisher Springfield Ill 1952
502 Just E E The Biology of the Cell Surface P Blakiston s Son & Company Philadelphia 1939
503 Kadatz R Arch exper Path u Pharmacol 207 363 1949 (Biol Abst 25 #?3?52)
504 Kahlson G Histamine edited by G E W Wolstenholme and C M O Connor p 248 Little Brown & Company Boston 1956
505 Kandletz M Jahrb Morphol Mikrosk Anat 60 469 1928 (Abst 1 Gebenbaur s Morphol Jahrb) (Biol Abst 5 #5876)
506 Kano K Cytologia 16 341 1952
507 Kapeller Adler R Histamine edited by G E W Wolstenholme and C M O Connor p 356 Little Brown & Company Boston 1956
508 Katz L N and Stamler J Experimental Atherosclerosis Charles C Thomas Publisher Springfield Ill 1953
509 Katzberg A A Anat Rec 118 393 1954 (#250)
510 Keetch M K Reed R and Wood M J J Path & Bact 71 4?7 1956
511 Kellicott W E A Textbook of General Embryology Henry Holt & Company New York 1913
512 Kelsall M A Organization and Developmental Modifications in the Mucosa of the Rabbit Appendix M A Thesis University of Colorado Boulder 1940
513 Kelsall M A J Morphol 70 115 1942
514 Kelsall M A Effects of Benzene Poisoning on the Intestinal Lymphoid Tissue of the Belgian Hare Ph D Thesis University of Colorado Boulder 1943
515 Kelsall M A Science 102(2653) 456 1945

667 McGovern V J J Path & Bact 72 143 1956
668 McHenry E W and Gavin G Biochem J 26 1365 1932
669 McIntire F C *Histamine* edited by G E W Wolstenholme and C M O Connor p 170 Little Brown & Company Boston 1956
670 McIntire F C *Histamine* edited by G E W Wolstenholme and C M O Connor p 416 Little Brown & Company Boston 1956
671 McKusick V A *Heritable Disorders of Connective Tissue* C V Mosby Company St Louis 1956
672 McManus J F A *Connective Tissue in Health and Disease* edited by G A boe Hansen p 31 Ejnar Munksgaards Forlag Copenhagen 1954
673 McMaster P D *The Nature and Significance of the Antibody Response* edited by A M Pappenheimer Jr p 13 Columbia University Press New York 1953
674 McNaught M L and Owen E C Biochem J 56 151 1954 (Biol Abst 28 #14958)
675 McNutt W S *Progress in the Chemistry of Organic Natural Products* edited by L Zechmeister p 401 Springer Verlag Wien 1952
676 Meiklejohn A P *Vitamins and Hormones Advances in Research and Applications* edited by R S Harris G F Marrian and K V Thimann Vol XI p 61 Academic Press Inc New York 1953
677 Meites J *Metabolism* 1 58 1952
678 Meites J and Feng Y S L Proc Soc Exper Biol & Med 85 341 1954
679 Meneghini P *Proc Third Internat Cong International Society of Hematology* edited by C V Moore p 505 Grune & Stratton Inc New York 1951
680 Meng H C and Davis W S A M A Arch Path (Sec II) 60 276 1955
681 Menkin V Proc Soc Exper Biol & Med 51 39 1942
682 Menkin V *Dynamics of Inflammation* The Macmillan Company New York 1950
683 Menkin V *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 137 Acta Inc Montreal 1953
684 Merck *The Merck Index of Chemicals and Drugs* Ed 6 Merck & Co Inc Rahway N J 1952
685 Meurman L Acta med scandinav 152 115 1955
686 Meyer K *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 1 0 Acta Inc Montreal 1953
687 Meyer K *Connective Tissue in Health and Disease* edited by G A boe Hansen p 54 Ejnar Munksgaards Forlag Copenhagen 1954
688 Meyer K H and Fellig J Experientia 6 186 1950
689 Meyer K H and Schwartz D E Helvet chim acta 33 1651 1950
690 Meyer A W J Med Research 31 51 1917
691 Michels N A Arch Path 11 775 1931
692 Michels N A Am J Anat 57 439 1935
693 Michels N A *Handbook of Hematology* edited by H Downey Vol I p 231 Paul B Hoeber Inc New York 1938
694 Michels N A *Blood Supply and Anatomy of the Upper Abdominal Organs* J B Lippincott Company Philadelphia 1955
695 Michels N A and Globus J Arch Path 8 371 1929
696 Miller C O Seibers A E and Brazda F G Proc Soc Exper Biol & Med 30 636 1933
697 Miller D K and Rhoads C P J Clin Inves 12 1009 1933 (Biol Abst 8 #17671)
698 Mirsky A E *Nucleic Acids and Nucleoproteins* Cold Spring Harbor Symp Quant Biol Vol 12 p 143

ogy of the Skin Ed 3 Paul B Hoeber Inc (med book department of Harper & Brothers) New York 1948

631 MADDOCK C I Proc Soc Exper Biol & Med 85: 598 1954

632 MADSEN H Nature London 143 981 1939

633 MALLORY F B *The Principles of Pathologic Histology* W B Saunders Company Philadelphia 1918

634 MANDL F J Internat Coll Surgeons 7 34 1944

635 MANN F C Endocrinology 3 299 1919

636 MANNA L AND HAUGE S M J Biol Chem 202 91 1953

637 MANSON L A J Bact 66 703 1953 (Biol Abst 28 #16335)

638 MARINE D *Special Cytology* Ed 2 edited by E V Cowdry Vol II p 797 Paul B Hoeber Inc New York 1932

639 MARINE D AND MANLEY O T J Lab & Clin Med 3 48 1917

640 MARINE D MANLEY O T AND BAUMANN L J J Exper Med 40 429 1924

641 MARONGUI M Policlinico (sez chir) 33 619 1926 (Biol Abst 2 #11195)

642 MARSHALL A H E J Path & Bact 65 29 1953

643 MARSHALL W Mississippi Valley M J 79 202 1957

644 MARSTON H R Ann Rev Biochem 8 557 1939

645 MARSTON H R Physiol Rev, 32 66 1952

646 MARTIN A P SHAW C R AND WILLIAMS H H J Nutrition 49 163 1953 (Biol Abst 27 #21650)

647 MARTIN G J Ann New York Acad Sc 61 646 1955

648 MARTIN G L *Biological Antagonism* Blakiston Company New York 1951

649 MARTIN H AND ROKA L Acta haemat 10 26 1953

650 MASON J C A quantitative study of the macroscopic lymphoid tissue in the small intestine cecum and appendix of normal rabbits Unpublished material 1947

651 MASON M F Surgery 6 451, 1939

652 MASSON P AND WOLF M Bull Assoc franç etude cancer 1920 331 1920

653 MATHEWS A P *General Cytology* edited by E V Cowdry p 15 University of Chicago Press Chicago 1924

654 MATHEWS M B AND DORFMAN A Physiol Rev 35 381 1955 (Biol Abst 30 #2196)

655 MAVOR M F AND GRECO A F Fed Proc 13 261 1954 (#866)

656 MAXIMOW A A *Special Cytology* edited by E V Cowdry p 319 Paul B Hoeber Inc New York 1928

657 MAXIMOW A A *Special Cytology* edited by E V Cowdry p 601 Paul B Hoeber Inc New York 1932

658 MAXIMOW A A AND BLOOM W *A Textbook of Histology* W B Saunders Company Philadelphia 1930

659 MAXIMOW A A AND BLOOM W *A Textbook of Histology* Ed 5 W B Saunders Company Philadelphia 1948

660 MAXIMOW A A AND BLOOM W *A Textbook of Histology* Ed 6 W B Saunders Company Philadelphia 1952

661 MAXIMOW A A AND BLOOM W *A Textbook of Histology* Ed 7 W B Saunders Company Philadelphia 1957

662 McCOY R H *The Rat in Laboratory Investigation* edited by J Q Griffith Jr and E J Farris p 67 J B Lippincott Company Philadelphia 1942

663 McCUTCHEON M *Pathology* Ed 2 edited by W A D Anderson p 13 C V Mosby Company St Louis 1953

664 McELEN C S SELYE H AND COLLIP J B Proc Soc Exper Biol & Med 36 390 1937

665 McGOVERN V J J Path & Bact 69 283 1955

666 McGOVERN V J J Path & Bact 71 1 1956

733 Najjar V A and Barrett R *Vitamins and Hormones* edited by R S Harris and K V Thimann Vol III p 2. Academic Press Inc New York 1945

734 Needham A E *Regeneration and Wound Healing* Methuen & Co Ltd London 1952

735 Needham J *Biochemistry and Morphogenesis* University Press Cambridge England 1942

736 Neel J V Bolt R J and Pollard H M Gastroenterology 26 1 1954 (Exper Med XIV 2 #4882)

737 Nehls K Zeitschr Zellforsch u mikrosk Anat 25 283 1936 (Biol Abst 11 #14796)

738 Nelson W O and Merckel C Proc Soc Exper Biol & Med 36 825 1937

739 Nemilov A V Arch Russ Anat Hist et Emb 15 40 (Germ) 167 1936 (Biol Abst 11 #13875)

740 Neuhold R Schweiz Ztschr allg Path 19 748 1956 (Excerpta Med XVI Cancer 5 #2619)

741 Newman H H *Evolution Genetics and Eugenics* Ed 3 University of Chicago Press Chicago 1935

742 Nichol C A Dietric L S Cravens W W and Elvehjem C A Proc Soc Exper Biol & Med 70 40 1949

743 Niggemann Fr B M Univ Colorado Stud 27: 95 1946

744 Nilzen A and Paldrok H Acta dermat venereol 30 318 1950

745 Noback C R and Montagna W Anat Rec 96 279 1946

746 Nonidez J F and Windle W F *Textbook of Histology* Ed 2 McGraw Hill Book Company Inc New York 1953

747 Novikoff A B Exper Cell Res Suppl 2: 123 1952

748 Ober W B and Edgecomb J H Cancer 7: 75 1954

749 Oberling C *The Riddle of Cancer* Printing 3 Yale University Press New Haven 1944

750 Oelkers H A Arch Exper Path u Pharmakol 161: 344 1931

751 Oertel H New York Med J July 6 1907

752 Ogilvie R E *Pathological Histology* Ed 2 The Williams & Wilkins Company Baltimore 1943

753 Oka A Japan J Exptl Med 10 203 1932 (Biol Abst 7 #18686)

754 Oliver J Bloom F and Mangieri C J Exper Med 86 107 1947

755 Olson M Sides E Clark W G and MacDonald R Proc Soc Exper Biol & Med 49 396 1943

756 Oncley J R *Blood Cells and Plasma Proteins* edited by J L Tullis p 180 Academic Press Inc New York 1953

757 Oota Y and Osawa S Experientia 10 254 1954

758 Opdyke D L Nuclear fragmentation of lymphocytes in normal hamsters and in those treated with adrenal cortical extract Unpublished work

759 Oppel A *Lehrbuch der Vergleichen den Mikroskopischen Anatomie der Virbeltiere* Zweiter Teil Schlund und darm Gustav Fischer Verlag Jena 1897

760 Osmond H *Neuropharmacology* edited by H A Abramson p 183 Josiah Macy Jr Foundation New York 1956

761 Osogoe B Anat Rec 107 193 1950

762 Ottaviani G Arch Biochim Ital 3 51 1931

763 Padawer J Anat Rec 118 402 1954 (#269)

764 Padawer J and Gordon A S Anat Rec 124 209 1956

765 Padawer J and Gordon A S Anat Rec 124 659 1956

766 Padawer J Zimmerman A M Gordon A S and Marsland D Proc Soc Exper Biol & Med 92 323 1956

767 Paff G H and Bloom F Anat Rec 104 45 1949

768 Paff G H Bloom F and Reilly C J Exper Med 86 117 1947

769 Paff G H Sugiura H T Bocher C A and Roth J S Anat Rec 114 499 1952

Biological Laboratory Cold Spring Harbor L I New York 1947

699 Mirsky A E and Pollister A W *Frontiers in Cytochemistry* (*Biol Symposia 10*) edited by H L Hoer p 217 Cattell Press Lancaster Pa 1943

700 Mirsky A E and Pollister A W J Gen Physiol 30 117 1946

701 Mirsky A E and Ris H J Gen Physiol 31 475 1950

702 Moise T S and Smith G H J Exper Med 40: 13 1924

703 Molinelli E A Compt rend Soc de Biol 95 821 1926 (Biol Abst 1 #2751)

704 Money W L Fager J and Rawson R W Cancer Res 12 206 1952

705 Mongar J L *Histamine* edited by G E W Wolstenholme and C M O Connor p 74 Little Brown & Company, Boston 1956

706 Monkhouse F C and Jaques L B J Lab & Clin Med 36: 782 1950

707 Montagna W Chase H B and Melaragno H P J Nat Cancer Inst 12 591 1951

708 Montagna W and Noback R R Anat Rec 100 536 1948

709 Montefoschi S Caryologia 4 145 1952 (Biol Abst 27 #5091)

710 Montemartini G Policlinico (sez chir) 36 70 1929 (Biol Abst 5 #13847)

711 Moon V H and Tersharovec A Proc Soc Exper Biol & Med 85 7 1954

712 Moore J J J Lab & Clin Med 3 283 1918

713 Moore R A *A Textbook of Pathology* W B Saunders Company Philadelphia 1947

714 Morgan T H *Experimental Embryology* Columbia University Press New York 1927

715 Morison J E *Foetal and Neonatal Pathology* C V Mosby Company St Louis 1952

716 Morosov B D Arkhiv Biologiche skikh Nauk (Arch Sci Biol) 39 87 1935 (Biol Abst 11 #17087)

717 Morosov B D, and Strigaynova A R *J Physiol et path gen 32 1148* 1931 (Biol Abst 10 #14139)

718 Morris R S and Boggs T R Arch Int Med 8 806 1911

719 Mosbach E H and King C G J Biol Chem 185: 491 1950

720 Mota I Recent knowledges on mast cell and basophil physiology Copy of a paper presented before the 3rd meeting of the Sociedade Brasileira de Hematologia e Hemoterapia Recife Brazil February 16 1954

721 Mota I Beraldo W T Ferri A G and Junqueira L C U *Histamine* G E W Wolstenholm and C M O Connor p 47 Little Brown & Company Boston 1956

722 Mota I., Beraldo W T and Junqueira L C U Proc Soc Exper *Biol & Med., 83 455 1953*

723 Moynihan G A *Abdominal operations* W B Saunders Company Philadelphia 1906

724 Mulligan R M *Syllabus of Human Neoplasms* Lea & Febiger Philadelphia 1951

725 Munoz J Proc Soc Exper Biol & Med *95 328 1957*

726 Munoz J and Greenwald M A Fed Proc 16: 427 1957

727 Murphy J B *The Lymphocyte in Resistance to Tissue Grafting Malignant Disease and Tuberculous Infection* Monograph No 21 Rockefeller Inst for Med Res New York 1926

728 Murphy J B and Sturm E J Exper Med 38 183 1923

729 Murray R G *Histopathology of Irradiation from External and Internal Sources* edited by W Bloom p 243 McGraw Hill Book Company New York 1948

730 Muthmann E Anatomische Hefte 48 67 1913

731 Nagato M Centralbl f allg Path u path Anat 43: 289 1928 (Biol Abst 5 #2769)

732 Najjar V A *Fat Metabolism* edited by V A Najjar p 3 Johns Hopkins Press Baltimore 1954

733 Najjar V A and Barrett R *Vitamins and Hormones* edited by R S Harris and K V Thimann Vol III p 23 Academic Press Inc., New York 1945
734 Needham A E *Regeneration and Wound Healing* Methuen & Co Ltd London 1952
735 Needham J *Biochemistry and Morphogenesis* University Press Cambridge England 1942
736 Neel J V Bolt R J and Pollard H M Gastroenterology 26 1 1954 (Exper Med XIV 2 #4882)
737 Nehls K Zeitschr Zellforsch u mikrosk Anat 25 283 1936 (Biol Abst 11 #14796)
738 Nelson W O and Merckel C Proc Soc Exper Biol & Med 36 825 1937
739 Nemilov A V Arch Russ Anat Hist et Emb 15 40 (Germ) 162 1936 (Biol Abst 11 #13875)
740 Neuhold R Schweiz Ztschr allg Path 19 748 1956 (Excerpta Med XVI Cancer 5 #2619)
741 Newman H H *Evolution Genetics and Eugenics* Ed 3 University of Chicago Press Chicago 1935
742 Nichol C A Dietric L S Cravens W W and Elvehjem C A Proc Soc Exper Biol & Med 70 40 1949
743 Niggemann Fr B M Univ Colorado Stud 27 95 1946
744 Nilzen A., and Paldrok H Acta dermat venereol 30 348 1950
745 Noback C R and Montagna W Anat Rec 96 279 1946
746 Nonidez J F and Windle W F *Textbook of Histology* Ed 2 McGraw Hill Book Company Inc New York 1953
747 Novikoff A B Exper Cell Res Suppl 2 123 1952
748 Ober W B and Edgecomb J H Cancer 7: 75 1954
749 Oberling C *The Riddle of Cancer* Printing 3 Yale University Press New Haven 1944
750 Oelkers H A Arch Exper Path u Pharmakol 161 344 1931
751 Oertel H New York Med J July 6 1907
752 Ogilvie R E *Pathological Histology* Ed 2 The Williams & Wilkins Company, Baltimore 1943
753 Oka A Japanese J Exptl Med 10 203 1932 (Biol Abst 7 #18686)
754 Oliver J Bloom F and Mangieri C J Exper Med 86 107 1947
755 Olson M Sides E Clark W G and MacDonald R Proc Soc Exper Biol & Med 49 396 1943
756 Oncley J R *Blood Cells and Plasma Proteins* edited by J L Tullis p 180 Academic Press Inc New York 1953
757 Oota Y and Osawa S Experientia 10 254 1954
758 Opdyke D L Nuclear fragmentation of lymphocytes in normal hamsters and in those treated with adrenal cortical extract Unpublished work
759 Oppel A *Lehrbuch der Vergleichen den Mikroskopischen Anatomie der Wirbeltiere* Zweiter Teil Schlund und darm Gustav Fischer Verlag Jena 1897
760 Osmond H *Neuropharmacology* edited by H A Abramson p 183 Josiah Macy Jr Foundation New York 1956
761 Osogoe B Anat Rec 107 193 1950
762 Ottaviani G Arch Biochim Ital., 3 51 1931
763 Padawer J Anat Rec 118: 402 1954 (#269)
764 Padawer J and Gordon A S Anat Rec 124 209 1956
765 Padawer J and Gordon A S Anat Rec 124 659 1956
766 Padawer J Zimmerman A M Gordon A S and Marsland D Proc Soc Exper Biol & Med., 92: 328 1956
767 Paff G H and Bloom F Anat Rec 104 45 1949
768 Paff G H Bloom F., and Reily C J Exper Med 86 117 1947
769 Paff G H Sugiura H T., Bocher C A and Roth J S Anat Rec 114 499 1952

770 PAGE I H J Pharmacol & Exper Therap **105** 58 1952

771 PAGE I H Physiol Rev **34** 563 1954

772 PALADE G E Anat Rec **114** 427 1952

773 PALADE G E Anat Rec **118:** 433 1954

774 PALES L Ann anat path **11** 563 1934

775 PALITZ L L Am J Physiol **125** 607 1939 (Biol Abst 13 #7370)

776 PALMER L S AND KENNEDY C J Biol Chem **75** 619 1927 (Biol Abst 3 #6192)

777 PALOS L A Experientia **5** 207 1949

778 PAPPENHEIMER A M J Exper Med **20** 477 1914

779 PARDEE A B AND WILLIAMS I Arch Biochem **40:** 222 1952 (Biol Abst 27 #10272)

780 PARKER G H Anat Rec **26** 303 1923

781 PARODI U AND SCHILLACI G Boll Soc ital biol sper **2** 592 1927 (Biol Abst 4 #13059)

782 PARROT J L *Histamine* edited by G E W Wolstenholme and C M O Connor p 280 Little Brown & Company Boston 1956

783 PARROT J L AND LABORDE C *Histamine* edited by G E W Wolstenholme and C M O Connor p 57 Little Brown & Company Boston 1956

784 PASSALACQUA L A Bol Asoc med Puerto Rico **24** 221 1932 (Biol Abst 10 #1915)

785 PATON W D M *Histamine* edited by G E W Wolstenholme and C M O Connor p 59 Little Brown & Company Boston 1956

786 PATT H M AND BRUES A M *Radiation Biology* edited by A Hollaender Vol I Part 2 McGraw Hill Book Company Inc New York 1954

787 PATTERSON J F Quart Rev Biol **2** 399 1927

788 PAYNE A H KELLY L S AND JONES H B Cancer Res **12** 666 1952

789 PEARSE A G E J Clin Path **2** 81 1949

790 PEARSE A G E *Histochemistry Theoretical and Applied* Little Brown & Company Boston 1953

791 PEARSE R H AND WATSON E M Canadian J Research **E27** 43 1949

792 PEARSON K *Tables for Statisticians and Biometricians* Ed 3 Cambridge England 1945

793 PECK H M AND HOERR N L Anat Rec **109** 479 1951

794 PENNYPACKER M I J Morphol **66** 481 1940

795 PEREZ TAMAYO R AND IHNEN M *Am J Path* **29** 233 1953

796 PERLMAN P L AND CASSIDY J W Proc Soc Exper Biol & Med **83** 674 1953 (Biol Abst 28 #8457)

797 PERRY W L M *Histamine* edited by G E W Wolstenholme and C M O Connor p 242 Little Brown & Company Boston 1956

798 PETER H Ztschr Anat **104** 295 1935

799 PETERSON R R AND WEISS J M Anat Rec **118** 341 1954 (#47)

800 PETERSON W H AND PETERSON M S Bact Rev **9** 49 1945 (Biol Abst 20 #6828)

801 PINCUS P Brit M J **1949(4623)** 358 1949 (Biol Abst 24 #18676)

802 PINKERTON H *Pathology* Ed 2 edited by W A D Anderson p 407 C V Mosby Company St Louis 1953

803 PLAGGE J C Proc Soc Exper Biol & Med **44** 57 1940

804 PLAGGE J C J Morphol **68** 519 1941

805 PLAGGE J C Anat Rec **116** 237 1953

806 PLOUGH H H Wilhelm Roux Arch f Entwicklungsm d Organ (Abt D Zeitschr f wiss Biol) **115** 380 1929

807 POLLISTER A W Exper Cell Res (Suppl 2): 59 1952

808 POLLISTER A W AND LEUCHTENBERGER C Nature London **163:** 360 1949

809 POLLISTER A W, AND RIS H *Nucleic Acids and Nucleoproteins Cold Spring Harbor Symposia on Quant Biol* Vol XII p 147 Biological Laboratory Cold Spring Harbor L I New York 1947
810 PONDER E *A Textbook of Physiology* edited by J F Fulton p 598 W B Saunders Company Philadelphia 1949
811 PORTER R M *The Role of Plasma Cells in the Production of Globulin within the Mammary Gland and Time Studies on Antibody Response from Experimentally Induced Inflammation of the Udder* Ph.D Thesis University of Minnesota 1951
812 PRINCIGALLI S Alteneo Parmense 5 29? 1933 (Biol Abst 9 #6851)
813 PRINZMETAL M ORNITZ E M JR SIMKIN B AND BERGMAN H C Am J Physiol 152 48 1948
814 PULLINGER B D AND FLOREY H W J Path & Bact 45 157 1937
815 PURVES H D AND GRIESBACH W E Endocrinology 49 653 1951
816 PUTNAM F W J Cell & Comp Physiol 47 17 1956
817 PUTNAM F W AND MIYAKE A Arch Biochem 65 39 1956
818 PUTZU DONEDDU F Riv ital ginec 17 1 1934 (Biol Abst 10 #21087)
819 QUICK A J J Biol Chem 100 441 1933
820 QUIGLEY J P *Diseases of the Digestive System* Ed 3 edited by S A Portis p 155 Lea & Febiger Philadelphia 1953
821 RABINOVITCH M AND ANDREUCCI D Blood 4 580 1949
822 RABSON S M Am J Clin Path 19 393 1949
823 RAFFELL S *Immunity Hypersensitivity Serology* Appleton-Century Crofts Inc New York 1953
824 RAGAN C Comroe's *Arthritis and Allied Conditions* edited by J L Hollander p 123 Lea & Febiger Philadelphia 1953
825 RAGAN C *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 262 E nar Munksgaards Forlag Copenhagen 1954
826 RAGAN C HOWES E L PLOTZ C M MEYER K BLUNT J W AND LATTES R Bull New York Acad Med 26 251 1950
827 RAND M AND REID G Nature London 168 385 1951
828 RANDALL J T (ed) *Nature and Structure of Collagen* Academic Press Inc New York 1953
829 RANDOLPH T G AND ROLLINS J P *Proc First Clinical ACTH Conference* edited by J R Mote p 1 Blakiston Company Philadelphia 1950
830 RAPPORT M M GREEN A A AND PAGE I H Science 108 329 1948
831 RAPPORT M M AND KOELLE G B Arch internat pharmacodyn 92 464 1953 (Biol Abst 27 #2 711)
832 RASMUSSEN A T *Special Cytology* edited by E V Cowdry p 1675 Paul B Hoeber Inc New York 1932
833 RAY G B Am J Physiol 86 158 1928
834 REBUCK J W J Lab & Clin Med 32 660 1947
835 REICHARD P AND LAGERKVIST U Acta Chem scandinav 7 1207 1953 (Biol Abst 28 #10526)
836 REID E Cancer Res 14 249 1954
837 REID G AND RAND M Australian J Exper Biol & M Sc 29 401 1952 (Biol Abst 26 #21331)
838 REIMANN H A AND DEBERARDINIS C T Blood 4 1109 1949
839 REIMANN S P AND HANKELE E R Arch Path 17 ?04 1934 (Am J Cancer 22 685)
840 RENKIN E M Am J Physiol 168 538 1952
841 REUSE J J *Histamine* edited by G E W Wolstenholme and C M O Connor p 150 Little Brown & Company Boston 1956
842 RHOADS J E FLEIGELMAN M T AND PANZER L M J A M A 118 21 1942
843 RICE F A H Science 124 275 1956

844 RICHARDS M B Brit J Nutrition 3 109 1949

845 RICHTER G W J Exper Med 96 331 1952

846 RICHTER K M Ann New York Acad Sc 59 863 1955

847 RICHTER M N *Pathology* edited by W A D Anderson p 915 C V Mosby Company St Louis 1953

848 RIDDLE O Am J Physiol 68 557 1924

849 RIGDON R H *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 125 Acta Inc Montreal 1953

850 RILEY J F Science 118(3061) 332 1953

851 RILEY J F J Path & Bact 65 471 1953

852 RILEY J F J Path & Bact 65 461 1953

853 RILEY J F Blood 9 1123 1954

854 RILEY J F *Histamine* edited by G E W Wolstenholme and C M O Connor p 45 Little Brown & Company Boston 1956

855 RILEY J F *Histamine* edited by G E W Wol tenholme and C M O Connor p 398 Little Brown & Company Boston 1956

856 RILEY J F AND WEST G B J Physiol 120 528 1953

857 RILEY J F AND WEST G B J Path & Bact 69 269 1955

858 RINDANI T H *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 103 Acta Inc Montreal 1953

859 RINEHART J F *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 239 Ejnar Munks gaards Forlog Copenhagen 1954

860 RINEHART J F Ann New York Acad Sc 61 684 1955

861 RINGOEN A R *Handbook of Hematology* edited by H Downey Vol I p 149 Paul B Hoeber Inc New York 1938

862 RIS H AND KLEINFIELD R Chromosoma 5 363 1952 (Biol Abst 27 #21229)

863 ROBB-SMITH A H T *Nature and Structure of Collagen* edited by J T Randall p 14 Academic Press Inc New York 1953

864 ROBB-SMITH A H T *Connective Tissue in Health and Disease* edited by G Asboe Han en p 15 Ejnar Munk gaards Forlog Copenhagen 1954

865 ROBINSON W J Bone & Joint Surg 17 267 1935 (Biol Ab t 11 #1057)

866 ROBSON J M TROUNCE J R AND DIDCOCK K A H J Endocrinol 10 129 1953 (Biol Ab t 28 #15854)

867 ROCHA E SILVA M *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 237 Acta Inc Montreal 1953

868 ROCHA E SILVA M *Histamine Its Role in Anaphylaxis and Allergy* Charles C Thomas Publisher Springfield Ill 1955

869 ROCHA E SILVA M *Histamine* edited by G E W Wolstenholme and C M O Connor p 124 Little Brown & Company Boston 1956

870 ROCHE J *The Enzymes Chemistry and Mechanism of Action* edited by J B Sumner and K Myrbach p 473 Academic Press Inc New York 1950

871 ROCHE M HILLS A G AND THORN G W *Proceedings First Clinical ACTH Conference* J R Mote p 55 Blakiston Company Philadelphia 1950

872 RODIN IA N Klin Zt chr Univ Saratow 7 215 1929 (Biol Ab t 6 #4143)

873 RODNAN G P EBAUGH F G JR AND FOX M R S *Blood 12 355* 1957 (Biol Abst 31 #31200)

874 ROMANI J D Compt rend Soc de biol 147 391 1953

875 ROPES M W AND BAUER W *Synovial Fluid Changes in Joint Disease* Harvard University Press Cambridge Mass 1953

876 ROSAHN P D AND CASEY A E Am J M Sc 192 456 1936

877 Roscoe M H J Hyg 27 103 1931 (Biol Abst 3 #6119)
878 Rocke B *The Mechanism of Inflammation* edited by G Jasmin and A Roberts p 247 Acta Inc Montreal 1953
879 Rosenman R H, Solomon B Byers S, and Friedman M Proc Soc Exper Biol & Med 86 599 1954
880 Rose M H and Ely J O J Cell & Comp Physiol 34 71 1949
881 Rossiter R J *Neurochemistry The Chemical Dynamics of Brain and Nerve* edited by K A C Elliott I H Page and J H Quastel p 11 Charles C Thomas Publisher Springfield Ill 1955
882 Rothman S *Physiology and Biochemistry of the Skin* University of Chicago Press Chicago 1954
883 Rowley D A J Immunol 64 289 1950
884 Rowley D A and Benditt E P J Exper Med 103 399 1956
885 Rydh Ehrensvard I and Schmidt G Hoppe Seylers Ztschr physiol Chem 227 177 1934 (Biol Abst 9 #13741)
886 Sabin F R *Special Cytology* Ed 2 edited by E V Cowdry Vol I p 506 Paul B Hoeber Inc New York 1932
887 Sabin F R J Exper Med 70 189 1939
888 Sackler R R Fed Proc 11(1) 387 1952
889 Sadum E H Keith C K Pankey M J and Totter J R Am J Hyg 51 274 1949
890 Saller K Ztschr wissensch Biol Abt D Roux Arch Entwcklngs mechn d Organ 124: 298 1931 (Biol Abst 7 #9396)
891 Salzer M Ransohoff J L and Blatt H Ann Int Med 22 271 1945 (Biol Abst 19 #18711)
892 Samuels L T and Reich H *Annual Review of Biochemistry* edited by J M Luck Vol 21 p 129 Annual Reviews Inc Stanford Calif 1952
893 Sanders A G Florey H W and Barnes J M Brit J Exper Path 21 254 1940
894 Sandstrom C J Anat Rec 52 69 1932
895 Sawin P B *The Care and Breeding of Laboratory Animals* edited by E J Farris p 153 John Wiley & Sons Inc New York 1950
896 Schachter M *Histamine* edited by G E W Wolstenholme and C M O Connor p 167 Little Brown & Company Boston 1956
897 Schachter M Trefce E I and DeLamater E D Exper Cell Res 6 361 1954
898 Schafer E A *The Essentials of Histology* Lea Brothers & Company Philadelphia 1885
899 Schayer R W *Histamine* edited by G E W Wolstenholme and C M O Connor p 183 Little Brown & Company Boston 1956
900 Schayer R W *Histamine* edited by G E W Wolstenholme and C M O Connor p 298 Little Brown & Company Boston 1956
901 Schenken J R, and Burns E L *Pathology* Ed 2 edited by W A D Anderson p 760 C V Mosby Company St Louis 1953
902 Scheunert A and Krzywanek Fr W Pflugers Arch ges Physiol 213 198 1926 (Biol Abst 2 #9864)
903 Scheunert A and Krzywanek Fr W Pflugers Arch ges Physiol 221: 435 1929 (Biol Abst 5 #27540)
904 Schild H O *Histamine* edited by G E W Wolstenholme and C M O Connor p 139 Little Brown & Company Boston 1956
905 Schiodt E Acta med scandinav 95 49 1938
906 Schmitt F O Gross J, and Highberger J H Exper Cell Res Suppl 3 326 1955
907 Schneider J A and Yonkman F F Am J Physiol, 174 127 1954 (Biol Abst 28 #1041)

844 Richards M B Brit J Nutrition 3: 109 1949
845 Richter G W J Exper Med 96 331 1952
846 Richter K M Ann New York Acad Sc 59 863 1955
847 Richter M N *Pathology* edited by W A D Anderson p 915 C V Mosby Company St Louis 1953
848 Riddle O Am J Physiol 68 557 1924
849 Rigdon R H *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 125 Acta Inc Montreal 1953
850 Riley J F Science 118(3064) 332 1953
851 Riley J F J Path & Bact 65 471 1953
852 Riley J F J Path & Bact 65 461 1953
853 Riley J F Blood 9 1123 1954
854 Riley J F *Histamine* edited by G E W Wolstenholme and C M O Connor p 45 Little Brown & Company Boston 1956
855 Riley J F *Histamine* edited by G E W Wolstenholme and C M O Connor p 398 Little Brown & Company Boston 1956
856 Riley J F and West G B J Physiol 120 528 1953
857 Riley J F and West G B J Path & Bact 69 269 1955
858 Rindani T H *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 103 Acta Inc Montreal 1953
859 Rinehart J F *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 239 Ejnar Munksgaards Forlog Copenhagen 1954
860 Rinehart J F Ann New York Acad S 61 684 1955
861 Ringoen A R *Handbook of Hematology* edited by H Downey Vol I p 179 Paul B Hoeber Inc New York 1938
862 Ris H and Kleinfield R Chromosoma 5 363 1952 (Biol Abst 27 #21229)
863 Robb-Smith A H T *Nature and Structure of Collagen* edited by J T Randall p 14 Academic Press Inc New York 1953
864 Robb-Smith A H T *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 15 Ejnar Munksgaards Forlog Copenhagen 1954
865 Robinson W J Bone & Joint Surg 17 267 1935 (Biol Abst 11 #1057)
866 Robson J M, Trounce J R and Didcock K A H J Endocrinol 10 129 1953 (Biol Abst 28 #15854)
867 Rocha e Silva M *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 237 Acta Inc Montreal 1953
868 Rocha e Silva M *Histamine Its Role in Anaphylaxis and Allergy* Charles C Thomas Publisher Springfield Ill 1955
869 Rocha e Silva M *Histamine* edited by G E W Wolstenholme and C M O Connor p 124 Little Brown & Company Boston 1956
870 Roche J *The Enzymes Chemistry and Mechanism of Action* edited by J B Sumner and K Myrbach p 473 Academic Press Inc New York 1950
871 Roche M Hills A G and Thorn G W *Proceedings First Clinical ACTH Conference* J R Mote p 55 Blakiston Company Philadelphia 1950
872 Rodin Ia N Khn Ztschr Univ Saratow 7 215 1929 (Biol Abst 6 #4143)
873 Rodnan G P Ebaugh F G Jr and Fox M R S Blood 12 355 1957 (Biol Abst 31 #31700)
874 Romani J D Compt rend Soc de biol 147 391 1953
875 Ropes M W and Bauer W *Synovial Fluid Changes in Joint Disease* Harvard University Press Cambridge Mass 1953
876 Rosahn P D and Casey A E Am J M Sc 192 456 1936

877 Roscoe M H J Hyg 27 103 1931 (Biol Ab t 3 #6119)
878 Rose B *The Mechanism of Inflammation* edited by G Jasmin and A Roberts p 247 Acta Inc Montreal 1953
879 Rosenman R H Solomon B Byers S and Friedman M Proc Soc Exper Biol & Med 86 599 1954
880 Rose M H and Ely J O J Cell & Comp Physiol 34 71 1949
881 Rossiter R J *Neurochemistry The Chemical Dynamics of Brain and Nerve* edited by K A C Elliott I H Page and J H Quastel p 11 Charles C Thomas Publisher Springfield Ill 1955
882 Rothman S *Physiology and Biochemistry of the Skin* University of Chicago Press Chicago 1954
883 Rowley D A J Immunol 64 289 1950
884 Rowley D A and Benditt E P J Exper Med 103 399 1956
885 Rydh Ehrensvard I and Schmidt G Hoppe Seylers Ztschr physiol Chem 227 177 1934 (Biol Abst 9 #13741)
886 Sabin F R *Special Cytology* Ed 2 edited by E V Cowdry Vol I p 506 Paul B Hoeber Inc New York 1932
887 Sabin F R J Exper Med 70 189 1939
888 Sackler R R Fed Proc 11(1) 387 1952
889 Sadum E H Keith C K Pankey M J and Totter J R Am J Hyg 51 274 1949
890 Saller K Ztschr wissensch Biol Abt D Roux Arch Entwcklngs mechn d Organ 124 298 1931 (Biol Abst 7 #9396)
891 Salzer M Ransohoff J L and Blatt H Ann Int Med 22 271 1945 (Biol Abst 19 #18711)
892 Samuels L T and Reich H *Annual Review of Biochemistry* edited by J M Luck Vol 21 p 129 Annual Reviews Inc Stanford Calif 1952
893 Sanders A G Florey H W and Barnes J M Brit J Exper Path 21 254 1940
894 Sandstrom C J Anat Rec 52 69 1932
895 Sawin P B *The Care and Breeding of Laboratory Animals* edited by E J Farris p 153 John Wiley & Sons Inc New York 1950
896 Schachter M *Histamine* edited by G E W Wolstenholme and C M O Connor p 167 Little Brown & Company Boston 1956
897 Schachter M Treece E L and DeLamater E D Exper Cell Res 6 361 1954
898 Schafer E A *The Essentials of Histology* Lea Brothers & Company Philadelphia 1885
899 Schayer R W *Histamine* edited by G E W Wolstenholme and C M O Connor p 183 Little Brown & Company Boston 1956
900 Schayer R W *Histamine* edited by G E W Wolstenholme and C M O Connor p 298 Little Brown & Company Boston 1956
901 Schenken J R and Burns E L *Pathology* Ed 2 edited by W A D Anderson p 760 C V Mosby Company St Louis 1953
902 Scheunert A and Krzywanek Fr W Pflugers Arch ges Physiol 213 198 1926 (Biol Abst 2 #9864)
903 Scheunert A and Krzywanek Fr W Pflugers Arch ges Physiol 221 435 1929 (Biol Abst 5 #27540)
904 Schild H O *Histamine* edited by G E W Wolstenholme and C M O Connor p 139 Little Brown & Company Boston 1956
905 Schiodt E Acta med scandinav 95 49 1938
906 Schmitt F O Gross J and Highberger J H Exper Cell Res Suppl 3 326 1955
907 Schneider J A and Yonkman F F Am J Physiol 174 127 1954 (Biol Abst 28 #1044)

908 SCHOENHEIMER R *The Dynamic State of Body Constituents* Harvard University Press Cambridge Mass, 1942
909 SCHOFIELD B M AND WALKER M M J Physiol **122** 487 1953 (Biol Abst 28 #18032)
910 SCHRADER F AND LEUCHTENBERGER C Exper Cell Res **3** 136 1952
911 *SCHRIDDE H Pathologische Anatomie* Bd 2 L Aschoff Hrsg p 101 Gustav Fischer Verlag Jena 1911
912 SCHUBERT M AND HAMERMAN D J Histochem **4** 159 1956
913 SCHULTZ J CASPERSSON T AND AQUILONIUS L *Proc Nat Acad Sc* **26** *515 1940*
914 SCHWARTZ C SCOTT E B AND FERGUSON R L Anat Rec **110** 313 1951
915 SCOTT E B AND SCHWARTZ C Proc Soc Exper Biol & Med, **84** 271 *1953*
916 SEABRA P Ann New York Acad Sc **59** 1092 1955
917 SEED W I *Nature and Structure of Collagen* edited by J T Randall p 250 Academic Press Inc New York 1953
918 SEIGEL G B AND WORLEY L G Anat Rec **111** 597 1951
919 SEIGEL J H Anat Rec **117** 583 1953 (#121)
920 SELBY C C *Analytical Cytology* edited by R C Mellors p 1 Blakiston Company (division of McGraw Hill Book Company Inc) New York 1955
921 SELYE H Brit J Exper Path **17** 231 1936
922 *SELYE H The Physiology and Pathology of Exposure to Stress* Acta Inc Montreal 1950
923 SELYE H *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 53 Acta Inc Montreal 1953
924 SELYE H *The Hypophyseal Growth Hormone Nature and Actions* edited by P W Smith O H Gaebler and C N H Long p 123 Blakiston Company (division of McGraw Hill Book Company Inc) New York 1955
925 SERRA J A Portugaliae Acta Biologica **2** 25 1947
926 SEVEREANU G Arch Anat u Entwicklng gesch **1909** 93 1909
927 SHANKLIN W M Anat Rec **111** *177 1951*
928 SHARP L W *Introduction to Cytology* Ed 3 McGraw Hill Book Company Inc New York *1934*
929 SHAW A F B AND SHAFI A J Path & Bact **45** 215 1937
930 SHAY H GERSHON COHEN J FELS S *MERANZE D* AND MERANZE T J A M A **112** 290 1939
931 SHERROD T R AND BOBB G A Fed Proc **16** 335 1957 (#1453)
932 SHORE B Proc Soc Exper Biol & Med **90** 415 1955
933 SHORE P A PLETSCHER A TOMICH E G *KUNTZMAN R AND BRODIE* B B J Pharmacol & Exper Therap **117** 232 1956
934 SHWARTZMAN G AND STRAUS L J Nutrition **38** 131 1949
935 SIERACKI J C Ann New York Acad Sc **59** 690 1955
936 SILBERBERG M AND SILBERBERG R Anat Rec **80** 317 1941
937 SILBERBERG M AND SILBERBERG R Anat Rec **102** 141 1948
938 SIMONCINI M Tumori *14 394 1940* (Biol Abst 16 #4369)
939 SIMPSON M E LI C H REINHARDT W O AND EVANS H M Proc Soc Exper Biol & Med **54** 135 1943
940 SIMPSON W L *Connective Tissue in Health and Disease* edited by G Asboe Hansen p 275 Ejnar Munksgaards Forlog Copenhagen 1954
941 SINHA Y K AND WEST G B J Pharm & Pharmacol **5** 370 1954 (Biol Abst 28 #6010)
942 SIRTORI C AND RUSSENBERGER G V Tumori **29** 13 1953 (Biol Abst 28 #3643)
943 SISSON S *The Anatomy of the Do*

mestic Animals Ed 2 W B Saunders Company Philadelphia 1921
944 SIZER I W AND BRINDLEY C O AND WAGLEY P F Internat Cong Bio chem 1 561 1949 (#287/8)
945 SJOERDSMA A WAALKES T P AND WEISSBACH H Science 125 1202 1957
946 SJOSTRAND T Skandinav Arch Physiol Suppl 71 1 1935 (Biol Abst 10 #18676)
947 SKELTON F R Canadian J Biochem & Physiol 34 520 1956 (Biol Abst 30 #28799)
948 SKOW R Endocrinology 51 42 1952
949 SLACK H G B *Nature and Structure of Collagen* edited by J T Randall p 51 Academic Press Inc New York 1953
950 SLACK H G B Ann Rheumat Dis 14 238 1955
951 SMELSER G K Endocrinology 34 29 1944
952 SMITH C THATCHER E C KRAEMER D Z AND HOLT E S J Morphol 91 199 1952
953 SMITH D J Fed Proc 12 133 1953 (#436)
954 SMITH R O AND WOOD W B J Exper Med 90 555 1949
955 SMITH S AND McCABE E S Ann Int Med 29 445 1948 (Blood 4 197)
956 SNIDER R S *Histopathology of Irradiation from External and Internal Sources* edited by W Bloom p 32 McGraw Hill Book Company Inc New York 1948
957 SNIDER P S AND RAPER J R *Effects of External Beta Radiation* edited by R E Zirkle p 152 McGraw Hill Book Company Inc New York 1951
958 SOBERON G Dissertation Abst 16 2070 1956
959 SOBOTTA J Anat Anz 77 184 1933
960 SOGNNAES R F *Histology* edited by R O Greep p 458 Blakiston Company New York 1954
961 SOKOLOFF B REDD J B AND DUTCHER R Quart J Florida Acad Sc 12 185 1950 (Biol Abst 25 #7446)
962 SOLARINO G AND CATTAINO V Boll Soc ital biol sper 5 985 1930 (Biol Abst 6 #18517)
963 SOLI U Arch ital de biol 52 215 1909
964 SOLLMANN T *A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology* Ed 4 W B Saunders Company Philadelphia 1932
965 SOMMERS I G *Histology and Histopathology of the Eye and Its Adnexa* Grune & Stratton Inc New York 1949
966 SOMMERS S C J Gerontol 11 251 1956
967 SORGE G Boll Soc ital biol sper 1 443 1926 (Biol Abst 2 #11226)
968 SOULA L C J phy iol et path gen 26 609 1928 (Biol Abst 4 #11330)
969 SOURKES T L *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder Vol I p 58 Academic Press Inc New York 1953
970 SPEIRS R S Ann New York Acad Sc 59 706 1955
971 SPOERLEIN M T AND MARCOLIN S Fed Proc 13 407 1954 (#1336)
972 SPURR C L Proc Soc Exper Biol & Med 64 259 1947 (Biol Abst 21 #20063)
973 SRIVASTAVA D S La Cellule 55 129 1952 (Biol Abst 27 #8504)
974 SSACHAROFF G P Zt chr ges exper Med 66 411 1929 (Biol Abst 4 #26606)
975 STACK M V *Nature and Structure of Collagen* edited by J T Randall p 124 Academic Press Inc New York 1953
976 STAVRAKY G J Pharmacol & Exper Therap 43 265 1931 (Biol Abst 7 #3428)
977 STEADMAN E AND STEADMAN EL *Nucleic Acid* Symposia No 1 Soc Exper Biol p 233 University Press Cambridge England 1951

978 STEFANINI M AND DAMESHEK W *Hemorrhagic Disorders A Clinical and Therapeutic Approach* Grune & Stratton Inc New York 1955

979 STEIN P Ztschr klin Med 108 566 1928 (Biol Abst 6 #21772)

980 STEINBERG B AND MARTIN R A J Immunol 52 71 1946

981 STENGEL A AND FOX H *A Textbook of Pathology* Ed 6 W B Saunders Company Philadelphia 1915

982 STERN K G Exper Cell Res Suppl 2 1 1952

983 STERN K AND WILLHEIM R *The Biochemistry of Malignant Tumors* Reference Press Brooklyn New York 1943

984 STEWART M O FLORIO L AND MUGRAGE E R *J Exper Med.,* 80 189 1944

985 STOERK H C AND ZUCKER T F Proc Soc Exper Biol & Med 56 151 1944 (Biol Abst 18 #21814)

986 STONES H H *Oral and Dental Diseases Aetiology Histopathology Clinical Features and Treatment* Ed 2 The Williams & Wilkins Company Baltimore 1951

987 STOWELL R D AND ZORZOLI A Stain Technol 51 51 1947

988 STROHL A BINET L AND FOURNIER B Compt rend Soc de biol 97 148 1927 (Biol Abst 2 #5342)

989 STRONG O S AND ELWYN A *Human Neuroanatomy* Ed 3 The Williams & Wilkins Company Baltimore 1953

990 STUDER A Experientia 10 148 1954

991 SUGIURA H T Anat Rec 118 561 1954

992 SUKUMARAN M AND BLOOM W L Proc Soc Exper Biol & Med 84 631 1953

993 SUMNER J B AND SOMERS G F *Chemistry and Methods of Enzymes* Ed 3 Academic Press Inc New York 1953

994 SUMNER R Length of rabbit intestines Biol News-Letter University of Colorado 8(2) 94 1935

995 SUNDBERG M Acta path et micro biol scandinav Suppl 107: 1 1955

996 SUNDBERG R D SCHAAR F E POWELL M J S AND DENLOER D Anat Rec 118 35 1954

997 SUOMALAINEN H O T Ann Zool Soc Zool Bot Fennicae 'Vanamo 16 1 1954 (Biol Abst 30 #25877)

998 SURGENOR D M HUNTER M J AND BROWN R *Blood Cells and Plasma Proteins* edited by J L Tullis p 315 Academic Press Inc New York 1953

999 SWIFT H *Internat Rev of Cytology* edited by G H Bourne and J F Danielli Academic Press Inc New York 1953

1000 SYLVEN B Exper Cell Res 1: 582 *1950*

1001 SYLVEN B Unio Internat Contra Cancrum ACTA 8 (No Special) 211 1952

1002 SZENT GYORGYI A *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 15 Acta Inc *Montreal 1953*

1003 SZENT GYORGYI A Ann New York Acad Sc 61 732 1955

1004 SZIRMAI J A J Histochem 4 96 1956

1005 TABOR H *Histamine* edited by G E W Wolstenholme and C M O Connor p 318 Little Brown & Company Boston 1956

1006 TAFFEL M AND HARVEY S C Proc Soc Exper Biol & Med 38: 518 1938

1007 TALBOT N B SOBEL E H McARTHUR J W AND CRAWFORD J D *Functional Endocrinology from Birth Through Adolescence* Harvard University Press Cambridge Mass 1952

1008 TALIAFERRO W H AND TALIAFERRO L G J Immunol 66 191 1951

1009 TARANTOLA R Pathologica 25 848 1933 (Biol Abst 10 #8074)

1010 TAUBENHAUS M *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 97 Acta Inc Montreal 1953

1011 TAUBENHAUS M JACOBSON M MOR

TON J V AND LEVINE R Proc Soc Exper Biol & Med 84 646 1953
1012 TAUBENHAUS M TAYLOR B AND MORTON J W Endocrinology 51 183 1952
1013 TAYLOR E L Vet Rec 52 259 1940 (Biol Abst 14 #10645)
1014 TAYLOR H C Arch Surg 21 412 and 597 1930
1015 TAYLOR J H Am Nat 91 209 1957 (Biol Abst 31 #36837)
1016 TERROINE E F Centre National de la Recherche Scientifique 13 Quai Anatole France Paris (VII) 1952
1017 TERRY T Proc New York Path Soc 17 42 1917
1018 THOMPSON W P Bull Johns Hopkins Hosp 51 365 1932 (Biol Abst 8 #11203)
1019 THYGESON P *Systemic Ophthalmology* edited by A Sorsby p 244 C V Mosby Company St Louis 1951
1020 TITUS E AND UDENFRIEND S Fed Proc 13 411 1954 (#1348)
1021 TODD J C AND SANFORD A H *Clinical Diagnosis by Laboratory Methods* Ed 6 W B Saunders Company Philadelphia 1927
1022 TOKUE K Tohoku J Exper Med 12 459 1929 (Biol Abst 5 #9109)
1023 TOMPKINS E A Ann New York Acad Sc 59 732 1955
1024 TONUTTI E *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 111 Acta Inc Montreal 1953
1025 TORYU Y Sci Rept Tohoku Imp Univ 4th Ser Biol 5 391 1930 (Biol Abst 6 #9558)
1026 TRAMONTANO V Pathologica 24 667 19 2 (Biol Abst 8 #5590)
1027 TRAUT E F *Rheumatic Diseases Diagnosis and Treatment* C V Mosby Company St Louis 1952
1028 TRENDELENBURG U *Histamine* edited by G E W Wolstenholme and C M O Connor p 278 Little Brown & Company Boston 1956
1029 TROILO E Compt rend Soc de biol 99 1008 1928
1030 TSCHASSOWNIKOW N Ztschr f Zellforsch u mikr Anat 8 251 1929
1031 TUBA J AND ROBINSON M I J Biol Chem 203 917 1953
1032 TULLIS J L *Blood Cells and Plasma Proteins Their State in Nature* edited by J L Tullis p 257 Academic Press Inc New York 1953
1033 TUR J Compt rend Soc de biol Paris 97 473 1927
1034 TUTKEWITSCH L Biochem Ztschr 198 47 1928 (Biol Abst 3 #5881)
1035 TWAROG B M AND PAGE I H Am J Physiol 175 157 1953 (Biol Abst 28 #17831)
1036 UDENFRIEND S BOGDANSKI D F AND WEISSBACH H Fed Proc 15 493 1956 (#1605)
1037 UDENFRIEND S CLARK C T AND TITUS E Experientia 8 379 1953 (Biol Abst 27 #22494)
1038 UDENFRIEND S AND TITUS E *Amino Acid Metabolism* edited by W D McElroy and H B Glass p 945 Johns Hopkins Press Baltimore 1955
1039 UDENFRIEND S AND WEISSBACH H Fed Proc 13 412 1954 (#1352) ✓
1040 UDENFRIEND S WEISSBACH H AND BOGDANSKI D F *Hormones Brain Function and Behavior* edited by H Hoagland p 147 Academic Press Inc New York 1957
1041 UMBREIT W W *Metabolic Maps* Burgess Publishing Company Minneapolis 1952
1042 UMBREIT W W *The Effect of ACTH and Cortisone upon Infection and Resistance* edited by G Shwartzman p 17 Columbia University Press New York 1953
1043 UNDERWOOD E J *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder p 426 Academic Press Inc New York 1953
1044 UNGAR G *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 151 Acta Inc Montreal 1953
1045 UNGAR G *Histamine* edited by G E W Wolstenholme and C M O Connor p 431 Little Brown & Company Boston 1956

1016 Uncley C C Brit J Nutrition 6 299 1952 (Biol Abst 27 #31986)

1017 Urbach E *Skin Diseases Nutrition and Metabolism* Grune & Stratton Inc New York 1946

1018 Urbach E and Gottlieb I M *Allergy* Ed 2 Grune & Stratton Inc New York 1949

1019 Uvnas B Physiol Rev 34 608 1954

1050 Vanotti A and Delachaux A *Iron Metabolism and Its Clinical Significance* translated by E Pulay Frederick Muller Ltd London 1942

1051 Varga L J Biol Chem 27 651 1955

1052 Vaughn J Spec Issue No 8 of Blood 8 1 1953

1053 Van De Velde J Compt rend Soc de Biol 97 1197 1927 (Biol Abst 3 #12615)

1054 Ventura S Fisiol e Med Rome 16 171 1948 (Biol Abst 25 #7463)

1055 Vercauteren R Enzymologia 16 1 1953

1056 Verzar F *The Suprarenal Cortex* edited by J M Yoffey p 39 Academic Press Inc New York 1953

1057 Viale C Rev med Rosario 18 329 1928 (Biol Abst 4 #21963)

1058 Vickery H B J Biol Chem 156 283 1944

1059 Vines H W C *Green's Manual of Pathology* Ed 17 The Williams & Wilkins Company Baltimore 1949

1060 Virchow R *Krankhaften Geschwulste Dreissig Vorlesungen* August *Hirschwald Berlin 1863*

1061 Vogt M and Bern H A Anat Rec 104 477 1949

1062 Voit K Sitzungsber Ges Beforderung Ges Naturwiss 4 113 1926 (Biol Abst 1 #7705)

1063 Von Euler U S *Histamine* edited by G E W Wolstenholme and C M O'Connor p 235 and 450 Little Brown & Company Boston 1956

1064 Von Horst D Acta haemat 10 342 1953

1065 Von Marbet R and Winterstein A Experientia 8 41 1952

1066 Waalkes T P and Weissbach H Proc Soc Exper Biol & Med 92 394 1956

1067 Wachstein M Ann New York Acad Sc 59 1052 1955

1068 Waksman S A and Davison W C *Enzymes* The Williams & Wilkins Company Baltimore 1926

1069 Waldschmidt Leitz E and Deutsch W Hoppe Seyler's Ztschr physiol Chem 167: 285 1927 (Biol Abst 3 #11791)

1070 Wang H Scheid H E and Schweigert B S Proc Soc Exper Biol & Med 85 382 1954

1071 Wangensteen O H *Diseases of the Digestive System* edited by S A Portis p 746 Lea & Febiger Philadelphia 1953

1072 Ward G E and Hendrick J W *Diagnosis and Treatment of Tumors of the Head and Neck* The Williams & Wilkins Company Baltimore 1950

1073 Wasastjerna C Blood (Analytical Rev) 8 1042 1953

1074 Waugh W G Brit M J 1 219 1940

1075 Webb M Exper Cell Res 5 27 1953

1076 Wegelius O Hjelmann G and Wasastjerna C Acta path et microbiol Scandinav 36 309 1955 (Biol Abst 30 #13129)

1077 Weicksel J Ztschr ges exper Med 50 415 1926 (Biol Abst 1 #9071)

1078 Weicksel J Ztschr ges exper Med 64 336 1929 (Biol Abst 4 #14602)

1079 Weiss L P *Histology* edited by R O Greep p 304 Blakiston Company New York 1954

1080 Weiss L P *Histology* edited by R O Greep p 364 Blakiston Company New York 1954

1081 Wells G C *Physiology and Biochemistry of the Skin* edited by S Rothman p 418 University of Chicago Press Chicago 1954

1082 Welsh J H Fed Proc **13** 162 1954 (#539)

1083 Wenzel D G and Rosenberg P J Am Pharm A (Scient Ed) **45** 1 1956

1084 West E S and Todd W R *Textbook of Biochemistry* The Macmillan Company New York 1952

1085 West G B *Histamine* edited by G E W Wolstenholme and C M O Connor p 14 Little Brown & Company Boston 1956

1086 West L S Anat Rec **28** 349 1924

1087 Whelan R F *Histamine* edited by G E W Wolstenholme and C M O Connor p 220 Little Brown & Company Boston 1956

1088 Whipple G H *Hemoglobin Plasma Protein and Cell Protein* Charles C Thomas Publisher Springfield Ill 1948

1089 Whistler R L and Smart C L *Polysaccharide Chemistry* Academic Press Inc New York 1953

1090 Whitfeld P R Australian J Biol Sci **6** 234 1953 (Biol Abst 28 #2882)

1091 Wichmann B E Acta path et microbiol Scandinav Suppl **108** 1 1955

109 Wiedersheim R *Grundriss der Vergleichenden Anatomie der Wirbelthiere* Ed 4 Gustav Fischer Verlag Jena 1898

1093 Wihman G Acta med scandinav **130(Suppl 205)** 1 1948

1094 Wilander O Skandinav Arch Physiol **81(Suppl 15)** 1 1939

1095 Wilhelmj C M Milani D P Meyers V W Gunderson D E Shuput D Racher E M and McCarthy H H Fed Proc **13** 165 1954 (#548)

1096 Williams F Proc Soc Exper Biol & Med **86** 77 1954

1097 Williams R J Am J Path **15** 377 1939 (Biol Abst 13 #13206)

1098 Williams W L Anat Rec **111** 699 1951

1099 Williams W L Lowe C U and Thomas L Anat Rec **115** 247 1953

1100 Williamson M B and Neumann G J Fed Proc **13** 322 1954 (#1065)

1101 Willis R A *The Principles of Pathology* C V Mosby Company St Louis 1950

1102 ✓ Wilson E B *The Cell in Development and Heredity* Ed 3 The Macmillan Company New York 1925

1103 Wintrobe M W *Clinical Hematology* Ed 2 Lea & Febiger Philadelphia 1947

1104 Winzler R J *Advances in Cancer Research* edited by J P Greenstein and A Haddow Vol I p 503 Academic Press Inc New York 1953

1105 Winzler R J *The Physiopathology of Cancer* edited by F Homburger and W H Fishman p 552 Paul B Hoeber Inc New York 1953

1106 Wiseman B K J A M A **103** 1524 1934

1107 Wislocki G B Bunting H and Dempsey F W Anat Rec **98** 527 1947

1108 Wislocki G B and Dempsey E W Anat Rec **96** 247 1946

1109 Wislocki G B Rheingold J J and Dempsey E W Blood **4** 562 1949

1110 Wissler R W Robson M J Fitch F Nelson W Jacobson L O J Immunol 70 349 1953 (Biol Abst 27 #25679)

1111 Woglom W H Arch Path & Lab Med **2** 533 and 709 1926

1112 Wolf G *Chemical Induction of Cancer* Harvard University Press Cambridge Mass 1952

1113 Wolfe J M and Hamilton J B Proc Soc Exper Biol & Med **36** 307 1937

1114 Wolf Heidegger G and Bejdl W Acta anat **19** 15 1953 (Biol Abst 28 #13325)

1115 Woolley D W *Hormones Brain Function and Behavior* edited by H Hoagland p 127 Academic Press Inc New York 1957

1046 UNGLEY C C Brit J Nutrition 6 299 1952 (Biol Abst 27 #31986)
1047 URBACH E *Skin Diseases Nutrition and Metabolism* Grune & Stratton Inc New York 1946
1048 URBACH E AND GOTTLIEB P M *Allergy* Ed 2 Grune & Stratton Inc New York 1949
1049 UVNAS B Physiol Rev 34 608 1954
1050 VANOTTI A AND DELACHAUX A *Iron Metabolism and Its Clinical Significance* translated by E Pulay Frederick Muller Ltd London 1949
1051 VARGA L J Biol Chem 217 651 1955
1052 VAUGHN J Spec Issue No 8 of Blood 8 1 1953
1053 VAN DE VELDE J Compt rend Soc de Biol 97 1197 1927 (Biol Abst 3 #12615)
1054 VENTURA S Fisiol e Med Rome 16 171 1948 (Biol Abst 25 #7463)
1055 VERCAUTEREN R Enzymologia 16 1 1953
1056 VERZAR F *The Suprarenal Cortex* edited by J M Yoffey p 39 Academic Press Inc New York 1953
1057 VIALE C Rev med Rosario 18 379 1928 (Biol Abst 4 #24963)
1058 VICKERY H B J Biol Chem 156 283 1944
1059 VINES H W C *Green's Manual of Pathology* Ed 17 The Williams & Wilkins Company Baltimore 1949
1060 VIRCHOW R *Krankhaften Geschwulste Dreissig Vorlesungen* August Hirschwald Berlin 1863
1061 VOGE M AND BERN H A Anat Rec 104 477 1949
1062 VOIT K Sitzungsber Ges Beforderung Ges Naturwiss 4 113 1926 (Biol Abst 1 #7205)
1063 VON EULER U S *Histamine* edited by G E W Wolstenholme and C M O Connor p 235 and 450 Little Brown & Company Boston 1956
1064 VON HORST D Acta haemat 10 312 1953
1065 VON MARBET R AND WINTERSTEIN A Experientia 8 41 1952
1066 WAALKES T P, AND WEISSBACH H Proc Soc Exper Biol & Med 92 394 1956
1067 WACHSTEIN M Ann New York Acad Sc 59 1052 1955
1068 WAKSMAN S A AND DAVISON W C *Enzymes* The Williams & Wilkins Company Baltimore 1926
1069 WALDSCHMIDT LEITZ E AND DEUTSCH W Hoppe Seyler s Ztschr physiol Chem 167 285 1927 (Biol Abst 3 #11791)
1070 WANG H SCHEID H E AND SCHWEIGERT B S Proc Soc Exper Biol & Med 85 382 1954
1071 WANGENSTEEN O H *Diseases of the Digestive System* edited by S A Portis p 746 Lea & Febiger Philadelphia 1953
1072 WARD G E AND HENDRICK J W *Diagnosis and Treatment of Tumors of the Head and Neck* The Williams & Wilkins Company Baltimore 1950
1073 WASASTJERNA C Blood (Analytical Rev) 8 1042 1953
1074 WAUGH W G Brit M J 1 219 1940
1075 WEBB M Exper Cell Res 5 ?? 1953
1076 WEGELIUS O HJELMANN G AND WASASTJERNA C Acta path et microbiol Scandinav 36 309 1955 (Biol Abst 30 #13129)
1077 WEICKSEL J Ztschr ges exper Med 50 415 1926 (Biol Abst 1 #9071)
1078 WEICKSEL J Ztschr ges exper Med 64 336 1929 (Biol Abst 4 #14602)
1079 WEISS L P *Histology* edited by R O Greep p 501 Blakiston Company New York 1954
1080 WEISS L P *Histology* edited by R O Greep p 364 Blakiston Company New York 1954
1081 WELLS G C *Physiology and Biochemistry of the Skin* edited by S Rothman p 418 University of Chicago Press Chicago 1954

INDEX

1116 Woolley D W and Shaw E J Biol Chem 203 69 1953 (Biol Abst 28 #13662)

1117 Woolley D W and Shaw E Fed Proc 13 325 1954 (#1074)

1118 Woolley D W and Shaw E S Fed Proc 14 307 1955 (#994)

1119 Worley L G Ann New York Acad Sc 47 1 1946

1120 Worley L G and Spater H W Quart J Micro Sc 93 413 1952

1121 Wyatt G R Exper Cell Res Suppl 2 201 1952

1122 Yamada E Acta anat 29 267 1957 (Biol Abst 31 #31233)

1123 Yamagiwa K und Ichikawa K Mitt a d med Fak d k Univ Kyushu Fukuoka 15(2) 295 1915

1124 Yamagiwa K und Ichikawa K Japan Ztschr Kreb forsch 10(4) 1 1916

1125 Yamagiwa K und Ichikawa K Mitt a d med Fak d k Univ Kyushu Fuokuoka 17 19 1917

1126 Yoffey J M Biol Rev (Cambridge Philos Soc) 25 314 1950

1127 Yoffey J M and Courtice F C *Lymphatics Lymph and Lymphoid Tissue* Harvard University Press Cambridge Mass 1956

1128 Yoffey J M Drinker C K Clark E R Clark E I Lewis W H Sanders A G Florey H W and Medawar J Brit M J 2 873 1940

1129 Yokoyama H O Stowell R E and Mathews R M Anat Rec 109 139 1951

1130 Youmans J B Ann New York Acad Sc 61 729 1955

1131 Young W and Freeman N K Proc Soc Exper Biol & Med 90 463 1955

1132 Zbar M J Cippes K B and Alterman S I Am J Clin Path 26 1180 1956

1133 Zeleny C Illinois Biol Monog 3 1 1916

1134 Zeller E A *Histamine* edited by G E W Wolstenholme and C M O Connor p 339 Little Brown & Company Boston 1956

1135 Zimmerman H M *The Role of Nutritional Deficiency in Nervous and Mental Disease* Vol 22 of Res Publ Assoc Nerv & Ment Dis p 51 The Williams & Wilkins Company Baltimore 1943

1136 Zondek H *The Diseases of the Endocrine Glands* Ed 4 (2nd English) The Williams & Wilkins Company Baltimore 1944

1137 Zucker T F and Zucker L M *Vitamins and Hormones* edited by R S Harris and K V Thimann p 1 Academic Press Inc New York 1950

1138 Zweifach B W Anat Rec 73 475 1939

1139 Zweifach B W *The Mechanism of Inflammation* edited by G Jasmin and A Robert p 77 and 275 Acta Inc Montreal 1953

1140 Zweifach B W Ann New York Acad Sc 61: 670 1955

1141 Zwemer R I *Biochemistry and Physiology of Nutrition* edited by G H Bourne and G W Kidder p 39 Academic Press Inc New York 1953

1142 Zypkin S M Frankfurt Ztschr Path 38 84 1929 (Biol Abst 7 #1072)

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